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THE EFFECTS OF DIET, ANORECTIC DRUGS AND CAFFEINE ON VARIOUS
CARDIOVASCULAR PARAMETERS IN THE RAT.

Submitted by FELICITY SUZANNE MARSHALL LEIGH

for the Degree of PhD
of the University of Bath

1988

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DEDICATED TO MY MOTHER, VALDA MARIE

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ABSTRACT

Obesity has been shown to be a major risk factor for cardiovascular disease. The main aim of this work was to investigate cardiovascular changes occurring in dietary obese rats compared with lean controls. Blood pressure and heart rate (using the tail cuff method) increased more rapidly and attained higher peak levels in the obese rats. Two 'in vitro' preparations were used to identify specific areas where obesity-related changes could occur. The isolated perfused mesenteric vasculature from obese rats gave significantly greater maximal responses to noradrenaline and 5-hydroxytryptamine. While, the isolated perfused hearts from obese rats showed significantly greater contractility responses to noradrenaline.

Secondly, the effects of weight loss, induced by either withdrawal of the palatable diet or administration of an anorectic drug (amphetamine, fenfluramine or mazindol) given in the presence of the palatable diet were investigated in the obese rat. Dietary restriction alone reduced 'in vivo' heart rate and blood pressure but different effects were seen with the three drugs. Fenfluramine caused the largest reduction in blood pressure, while amphetamine produced a moderate reduction and mazindol caused a small increase. Mazindol caused an increase in heart rate while all the other procedures resulted in reduction.

A dietary survey in volunteers showed that approximately 25% of subjects increased consumption of caffeine while dieting. The potential cardiovascular risk factor of caffeine was therefore investigated in lean and obese rats. The results showed, rather

surprisingly, that caffeine appeared to diminish the adverse cardiovascular effects seen in the dietary obese rats. Thus, in the lean and obese caffeine-treated rats the time-related increases in heart rate and blood pressure were reduced. The isolated perfused mesenteric vasculature showed smaller maximal responses to noradrenaline and the isolated hearts showed reduced contractility responses to noradrenaline in caffeine treated animals.

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Chapter 1 : INTRODUCTION

CHAPTER 1 : INTRODUCTION

1.1 GENERAL INTRODUCTION

Obesity is often associated with elevated blood pressure (Dustan, 1983; Velasquez & Hoffmann, 1985; Schmeider & Messerli, 1987), and both conditions predispose to excess cardiovascular morbidity (Van Itallie, 1985; Lavie & Messerli, 1986). The aim of this work was, in part, to study possible cardiovascular changes occurring during the development of obesity, which might contribute to this elevation in blood pressure.

Weight reduction has been shown to reduce blood pressure in both normotensive and hypertensive individuals (MacMahon, 1985; Reisin, 1986). The effects of weight reduction (using either dietary restriction or pharmacological treatment with anorectic drugs) on cardiovascular function of the rat formed the second part of this work.

In addition, another suggested cardiovascular risk factor, namely caffeine (Robertson et al, 1978; Freestone & Ramsay, 1982), was also investigated.

1.2 OBESITY

1.2.1 Definitions of obesity

Obesity can be described as a condition in which there is an increased amount of adipose tissue in the body compared to normal. However, this type of definition is purely descriptive and can not be used for comparing the degree of obesity between different people. The definition also requires careful consideration of what is

"normal" , as this will alter with age, sex, and race. Committees on both sides of the Atlantic (Bray, 1979; DHSS/MRC,1976) have defined the condition of obesity as present where the body weight exceeds 120% of the upper limit of the wide range of weights-for-height which were quoted by the Metropolitan Life Insurance Company (1960). Quetlet's Index or Body Mass Index (BMI) is commonly used to define obesity (Quetlet, 1869; Keys et al 1972). This value is calculated as $\text{Weight (kg)} / \text{Height}^2 (\text{m}^2)$. Garrow (1988) defined varying grades of obesity according to the range of BMI : Grade III, BMI >40 ; Grade II, BMI 30-40 ; Grade I, BMI 25-29.9 ; Grade 0, BMI 20-24.9 . The advantages of measuring obesity in this way are that the indices are straightforward to calculate and the procedures for measurement do not require technical knowledge or the use of sophisticated equipment. It is also important that the level of obesity determined for an individual should be independent of height, that is to say that two patients with the same degree of obesity should have the same BMI regardless of any differences in height. This is generally true for values of BMI.

The DHSS/MRC Group Report (1976) stated that, in spite of sources of variability, measurement of skinfold thickness may be regarded as a satisfactory practical index for community studies on the prevalence of obesity and its relationship to disease, and concluded that the method should be more widely used. The best established technique utilising skinfold thickness measurement is that using four sites (Durnin & Rahaman, 1967) : biceps, triceps, subscapular and supra-iliac. Three readings should be made at each site, or sufficient readings such that any three agree to within 2mm. The mean of the three readings is then calculated for each of the

four sites and the sum of these values entered into the table given by Durnin & Womersley (1974), in the column appropriate to the age and sex of the subject, to obtain a value for percentage body fat.

Obesity may also be determined by measurement of body composition. Densitometry (Behnke et al, 1942; Durnin & Rahaman, 1967), measurement of total body potassium (Boddy et al, 1976), measurement of total body water (Halliday & Miller, 1977) and measurement of body composition by neutron activation (Cohn et al, 1982) are all relatively accurate methods, used mainly to validate other less accurate methods of measurement. However, the technical knowledge and highly sophisticated (and expensive) equipment required, coupled with the time consuming feature of these methods, limits their application in routine clinical examination and epidemiological studies.

1.2.2 Aetiology and pathogenesis of obesity.

"Beware of those foods that tempt you to eat when you are not hungry and those liquors that tempt you to drink when you are not thirsty."

Socrates, 399 BC.

Recognition that obesity is related to excessive food intake is ages old and so is advice to the overweight, but obesity is more complex than simple overconsumption of food. A number of factors, either singly or in combination, may contribute to the development and maintenance of obesity.

1.2.2 a) Heredity

In some strains of animals obesity is an inherited trait, for example the ob/ob mouse and the fa/fa rat (see section 1.2.5 for

details of animal models of obesity). However, until recently there was little hard evidence that the same held true for humans. It was known that obese parents tend to have obese children but obesity in families was thought to be due more to their shared environment and lifestyle, including eating habits, than to heredity (Garn & Clark, 1976). However, recent studies using twins have provided support for a stronger genetic influence. Stunkard et al (1986a) convincingly demonstrated different concordance rates and correlation coefficients for monozygotic and dizygotic twins for various measures of overweight. Monozygotic twins raised in different homes were shown to be closer in weight than dizygotic twins raised together. Additional evidence for the genetic influence was described in an adoption study (Stunkard et al, 1986b) which found that obesity in adopted children correlated strongly with obesity in their natural parents (especially maternal obesity), whereas there was no significant correlation with obesity in their adoptive parents.

Another recent study also supports a genetic role in human obesity (Roberts et al, 1988). This study showed that 50% of the progeny of overweight mothers became overweight at some time during the twelve months of the study, as compared to none of the infants of lean mothers. The overweight infants were found to have a lower total energy expenditure than the lean infants. This reduced energy expenditure was thought to be an important factor in the rapid weight gain during the first year of life seen in infants born to overweight mothers.

Genetic factors may also give rise to a group of rare diseases known as 'dysmorphic' forms of obesity, including Prader-Willi syndrome, Bardet-Biedl syndrome, Cohen's syndrome and Carpenter's

syndrome. The obesity of the first two is generalised, while that of the second two is truncal-gluteal (Bray, 1987a). In most of these syndromes the obesity is only moderate, but in a few it may be pronounced. These forms of obesity are transmitted by recessive and dominant modes of inheritance. Prader-Willi syndrome is the most common disease of this group (Bray, 1987a).

Another genetic factor is that of body build or somatotype. Ectomorphs, thin individuals with long fingers, arms and legs, rarely become obese. In contrast, endomorphs, who are rounder individuals with larger abdomens than chests, have a greater likelihood of becoming obese. Mesomorphs, who tend to have a broad, muscular frame have a tendency to weight gain midway between the other two types (Kreutler, 1980).

1.2.2 b) Gestational, neonatal and infant nutrition.

Gestational maternal undernutrition in rats has been shown to predispose the offspring to obesity (Jones et al, 1984). However, overnutrition in rat pups during the first weeks of life, either by supplementing the diet of the dam during lactation (Roberts & Coward, 1985) or by reduction of the litter size (Hausberger & Volz, 1984) has been shown to cause the rat pups to become significantly heavier than controls. Earlier work by Winick et al (1972) demonstrated that varying food intake during their early development influenced the number and size of cells of various organs of rats.

Hirsch and Knittle (1970) showed that both number and size of adipose cells in obese children exceeded those of non-obese children. In 1980, Winick stated on the subject of infant nutrition that '...overnutrition, especially excess calorie intake may result in

accelerated cell division in adipose tissue and in a permanently hypercellular adipose depot. This is associated, in adult life, with an extremely refractory kind of obesity." This is sometimes referred to as the adipose cell theory. However this theory is not universally accepted.

Hausberger & Volz (1984) used rats to investigate the effects of early overnutrition and undernutrition upon subsequent development of obesity in adult life. Litters of 4, 9 and 24 pups were used. Pups from the small litters (4 pups) grew heavier and fatter than pups from control litters (9 pups) and pups from large litters (24 pups) remained small and stunted. However, after treatment with a high fat diet from 18-43 weeks of age, almost all the rats in all the groups became obese and the amount of adipose tissue (as percentage body weight) was essentially the same in all three fat-fed groups. Thus from this study it was deduced that early overfeeding does not predispose to obesity in later life and that underfeeding does not prevent or diminish the development of nutritional obesity or adipose tissue hypercellularity in rats. Therefore the adipose cell theory has been neither accepted nor rejected as important in the aetiology of human obesity at the present time.

1.2.2 c) Endocrine disorders.

In rare cases human obesity may be correlated with disturbances of the endocrine system. For example, an underactive thyroid gland may cause weight gain. Secondly, oestrogen and progesterone have been shown to affect food intake in female rats (Wade, 1975) and in women food intake is affected by the menstrual cycle (Dalvit-McPhillips, 1983; Kirby & Agadah, 1986). The latter study also showed that women

taking a combined oestrogen/progestogen contraceptive pill were significantly heavier than age-matched controls.

The role of insulin in food intake and body weight control is unclear. Some reports have postulated a role as a satiety hormone (Anika et al 1980). However, in other studies hyperphagic responses to insulin have been claimed (Lotter & Woods, 1977).

Glucocorticoid injections have been used to produce animal models of obesity (Hollifield, 1968). Clinical obesity due to glucocorticoid excess (Cushing's syndrome) is characterised by fat deposition in specific body areas (face, upper back, abdominal wall). Sodium and water retention also occur in this condition and this may account for some of the weight gain seen in this syndrome.

1.2.2 d) Metabolic disorders.

In experimental animals obesity is clearly associated with increased efficiency of energy storage (Bray & York, 1971; Bray & York, 1979). In most, if not all, forms of experimental obesity, the gain in body stores for a given quantity of food intake is higher than in correspondingly lean animals. Possible mechanisms by which such a difference in efficiency may occur are discussed below.

1) Futile cycles

Futile metabolic cycles, in which phosphorylated compounds are made from adenosine triphosphate (ATP) and then broken down, are of importance in insect biochemistry and also occur in mammalian biochemistry (Bray, 1987b). The quantitative importance of such futile cycles in the energetic efficiency of obesity, however, remains to be established.

ii) Low protein turnover

Forbes and Dresnick (1979) suggested that the obese individual loses nitrogen at a slower relative rate than the non-obese individual. This would reduce energy expenditure and thus allow additional energy for fat storage. Such effects have been shown in experimental animals (Shargill et al, 1984), though they have not yet been confirmed in man.

iii) Defective sodium pump.

Sodium pump activity in liver and skeletal muscle appears to be reduced in some obese experimental animals compared with lean controls (York et al, 1978). This metabolic derangement was proposed as one mechanism for the metabolic efficiency and enhanced fat storage of these genetically obese animals. The sodium pump from human erythrocytes has been reported to be reduced in obesity in one study (DeLuise et al, 1980) but most others have found no differences between lean and obese subjects (Simat et al, 1983). Moreover, human liver (Bray et al, 1981) and skeletal muscle (Charalambous et al, 1984) have shown a positive correlation between the sodium-pumping enzyme (Na^+, K^+ -ATPase) and body fat. A low activity of the sodium pump, therefore, does not appear to contribute as a cause of metabolic human obesity.

iv) Defective brown adipose tissue.

Brown adipose tissue (BAT) may play a role in the development of both genetic and dietary forms of obesity (Rothwell & Stock, 1979). In animals with genetic obesity, heat production by BAT is defective, and is not stimulated by injections of noradrenaline nor by electrical stimulation of the sympathetic nerves which supply the tissue (Himms-Hagen, 1979; Thurlby & Trayhurn, 1980). There is also

an important relationship between diet and BAT (see section 1.2.2 d) vii)). Some rats , when fed a cafeteria-style diet, gained less weight than anticipated from the amount of food eaten. The animals showed BAT hypertrophy and an increased energy expenditure following intravenous injections of noradrenaline (Rothwell & Stock 1979). These data led Rothwell and Stock (1979) to propose that during periods of dietary excess BAT might hypertrophy in an attempt to dissipate the food energy as heat rather than store it as fat.

Thermogenic beta-adrenoceptor agonist drugs such as BRL26830A were developed to stimulate thermogenesis in BAT as a possible mechanism for increasing energy expenditure and promoting weight loss (Arch et al, 1984a)). However, although the drug produced promising results in obese rodents (Arch et al, 1984a), by increasing energy expenditure and reducing body weight and body fat content, results of clinical trials in obese patients have not all shown significant weight loss (see section 1.2.4e)). Thus , although defective BAT and the resultant impairment of thermogenesis may play a role in the aetiology of human obesity, stimulation of the atypical beta-adrenoceptors present on BAT does not appear to always significantly improve thermogenesis and weight loss in obese humans.

v) Autonomic nervous system

Since the autonomic nervous system is involved in energy metabolism (Bray, 1984) it is conceivable that one or more subgroups of persons with idiopathic obesity have alterations in their autonomic nervous system that may promote obesity. Most of the present knowledge of autonomic function in obesity comes from animal studies. In several animal models of spontaneous obesity decreased sympathetic activity has been noted (Bray, 1987c).

A recent study of healthy men , aged 25-36 years, (Peterson et al, 1988) showed a weak but significant association between depressions in sympathetic and parasympathetic activity and increasing percentages of body fat. From these results it was suggested that autonomic alterations are important in human obesity as they are in animal models. It was proposed that a disordered homeostatic mechanism might promote excessive storage of energy by decreasing sympathetic activity, whilst defending against weight gain by decreasing parasympathetic activity.

vi) Increased lipoprotein lipase activity

Lipoprotein lipase, located along the endothelial surface is responsible for hydrolysis of the triglycerides in lipoproteins, thus making the fatty acids available for storage in fat cells. This 'gatekeeper' function puts it as an ideal locus to regulate the size of fat cells. In contrast with most other changes which revert to normal levels with weight loss , lipoprotein lipase activity increases with weight loss (Schwartz & Brunzell, 1981). The reason for this paradoxical change is unknown.

vii) Impaired dietary thermogenesis

The fact that metabolic rate rises with overeating was first noted in 1902 by a psychologist called Neumann (Hutton, 1985). He carried out an experiment on himself which involved eating about 2000 calories more than usual every day for several months. He found that although he gained weight to begin with, his weight soon stabilized at its new higher level and ceased to rise thereafter. This phenomenon was partly due to dietary thermogenesis. The work of Miller, Mumford and Stock (1967) showed that lean and obese subjects differ in the extent of their thermogenesis after food. They found that in lean subjects

overeating led to an increase of 20-50% in heat production, which tended to dissipate the excess intake. In obese subjects, however, the thermal dissipation of energy was less marked and thus the excess was more likely to be stored as fat. One explanation for the thermogenic response to a meal is that it results from enhanced activity in BAT (see section 1.2.2 d) iv)).

1.2.2 e) Other factors

1) Physical inactivity

Physical inactivity may contribute significantly to the development of some obesities (Stern, 1983). Although obese individuals are not always less active than those of normal weight, inactivity is often associated with obesity in persons of all ages. Rose and Mayer (1968), for example, reported that obese infants were considerably less active than those of normal weight. When obese adolescent girls in summer camp were observed using time lapse photography, they were found to be less active than normal weight girls (Bullen et al, 1964). In terms of routine daily activity, obese men and women walk less than normal weight adults (Chirico & Stunkard, 1960). It is not certain whether the inactivity is the cause or the result of the obesity in humans, though physical inactivity is known to promote obesity in normal lean rats (Ingle, 1949).

ii) Psychological and social factors

Before considering specific psychological or social factors it is first necessary to define the terms hunger and appetite. Garrow (1988) defined hunger as "a drive to eat which can be reliably caused in any normal animal or human subject by an energy deficit, which is removed when this energy deficit is abolished by feeding" and

appetite as "a drive to eat a food which is palatable in a particular situation".

There are significant differences between individuals in responsiveness to external food-related cues such as mealtime, sight of food, smell and taste of food (Rodin, 1980). Some individuals show a greater than normal response to these external food-related stimuli and consequently tend to eat more than others. A person may eat because the clock says that it is lunchtime, because other people are eating, because the food is "good for them" or simply because they like the food available rather than to satisfy hunger. Modern food advertising techniques depend upon providing a psychological stimulus to eat the food product concerned. Food is offered as a show of hospitality and it also traditionally accompanies social events, sports events, and even the working day.

Reports from New York (Goldblatt et al, 1965), London (Silverstone 1970) and Canada (Millar & Wigle, 1986) and other places agree that there is an inverse correlation between social class and prevalence of obesity in Western societies, this correlation being most consistently demonstrated for women.

Food tends to be used as a manipulative tool, particularly when dealing with children. Favourite foods are often used as rewards for good behaviour and withheld as punishment for mischief. Also, training a child to eat everything on the plate, regardless of feelings of fullness, may be responsible for setting up a lifelong pattern of overeating. The complex interactions between emotional factors and obesity are discussed in detail by Bruch (1973).

" A stomach full of food also soothes by draining the blood away from a disgruntled and maladaptive brain."

Selye, 1956.

The question of whether or not stress induces eating and whether food "soothes...a disgruntled ...brain" was debated by Robbins and Fray (1980). They stated that stressful stimuli do not induce eating 'per se' but that they might simply activate the organism making it more responsive to external food-related cues, thus directing behaviour towards eating. They also stated that they did not agree that eating could relieve feelings of stress. These opinions are not universally accepted, however. There is evidence that eating can alter mood. Lieberman et al (1986) presented a study which suggested that consumption of a high carbohydrate meal reduced feelings of depression in some subjects and induced feelings of depression in others. The "tail pinch" technique produces a hyperphagic animal model and is considered to be a model of stress-induced eating (Rowland & Antelman, 1976).

1.2.3 The hazards of obesity

a) Overall mortality

In 1959 the Build and Blood Pressure Study reported the mortality data of over four million life insurance policy holders (1935-1954) in the USA (Society of Actuaries, 1959). A U-shaped relationship was observed between relative body weight and the mortality ratio. Many scientists have criticised these data, which are biased by a number of factors. Firstly, the population sample from which the data were derived was not representative of the entire

USA population. Men were overrepresented and the people who insured themselves tended to be predominantly in the higher socio-economic classes. Secondly, the weights and heights were partly self-reported. Thirdly, only healthy people are given life insurance, thus chronically sick people were excluded from the study. In 1979 the Society of Actuaries published the results of a new Build Study on the relationship between body weight and mortality among life insurance policy holders 1952-1974 (Society of Actuaries,1979). This study showed the same relationship between relative weight and mortality as the 1959 study and it also provoked the same criticisms. In 1979 also, the American Cancer Society published the results of a study carried out from 1959-1972 in 26 states of the USA (Lew & Garfinkel,1979). The end point for the analysis was life or death per person (not per policy as in the previous two studies) and this is a more reliable criterion. The results were in good agreement with those of the 1959 Build and Blood Pressure Study.

The relationship between obesity and mortality depends upon age. Seltzer (1966) re-analysed the data of the Build and Blood Pressure Study. He found a curvilinear relationship between relative weight and mortality risk in younger people, but in people older than 50 years no such relationship was found. This is in agreement with results of other studies (Lew & Garfinkel,1979).

The causes of increased mortality due to overweight are numerous, ranging from traffic accidents to stroke and coronary heart disease. The relationship between obesity and mortality is also evident between obesity and some diseases and disorders (Royal College of Physicians,1983). Disorders for which this relationship applies include coronary heart disease, hypertension, diabetes

mellitus, gall bladder disease, arthritis, some cancers, and disorders of the respiratory system and the menstrual cycle. These are discussed further in sections 1.2.3 b) and 1.2.3 c). Some studies have found no relationship between obesity and mortality/morbidity (Borhani et al, 1965). However, these were usually performed in older aged populations (Larsson et al, 1981).

Recent studies suggest that the relationship between obesity and mortality/morbidity may depend upon the distribution of body fat, with those in whom the fat is predominantly located in the abdominal region (upper body segment obesity) being more at risk for some disorders and for death than persons with predominantly femoral-gluteal (lower body segment) obesity (Blair et al, 1984; Ducimetiere et al, 1986; Kalkhoff et al, 1983). The effect of fat distribution is independent of the degree of obesity (Kalkhoff et al, 1983). This may also explain, in part, why the relationship between obesity and mortality/morbidity is not evident in some studies. The greater overall risk of cardiovascular mortality seen in men compared to women may also be partially explained by this relationship, as body fat tends to accumulate in the upper body segment in men and in the lower body segment in women.

b) Cardiovascular risks

The relationship between excess body weight and increased risk of mortality and morbidity from cardiovascular disease is complex.

Observations of cardiovascular disease occurrence over 26 years in Framingham suggest that the degree of obesity is a significant and independent long-term predictor of such disease, particularly among women (Hubert et al, 1983). The risk of all forms of cardiovascular disease, excluding intermittent claudication, increased in both sexes

with the degree of obesity and this gradient was most pronounced in those subjects younger than 50 years of age. In this younger segment of the population, risk was 1.7 to 4.8 times greater in the heaviest compared to the leanest groups. Thus obesity has direct impact on the incidence of cardiovascular disease as an independent risk factor and has an influence on other risk factors such as hyperlipidaemia and hypertension. Hypertriglyceridaemia represents the most common form of hyperlipidaemia observed in the obese, with hypercholesterolaemia found less consistently (Nestel,1978). High density lipoprotein (HDL), purportedly inversely correlated with risk of developing cardiovascular disease, is decreased in the obese (Carlsson & Ericsson,1975).

There is a well-recognised association between the degree of overweight and blood pressure elevation (Boe et al 1957; Florey et al,1969; Tobian,1978). The Chicago People's Gas Company Study (Stamler,1967) was a cross-sectional examination of men aged 40-59 years, stratified according to the ratio of their observed weight to desirable weight at time of entry. As this ratio increased, there was a stepwise increase in the prevalence of diastolic hypertension (when either 95 or 100mmHg was used as the cut off point). A multivariate analysis of the effect of relative weight on blood pressure in more than 13000 men and women in the Chicago Board of Health Community Survey found a relationship between overweight and hypertension, which persisted when age, sex, race and family history of hypertension were taken into account (Stamler et al,1975).

In longitudinal studies, weight gain is associated with an increased risk of developing hypertension. The People's Gas Company Study (Stamler,1967) suggested that weight status at an early age and

the subsequent rapidity of weight gain are highly correlated with the development of hypertension in middle age. The Framingham Study (Kannel et al 1967) showed that subjects who were 20% overweight at time of admission had an eight times greater risk of developing hypertension than the group that were 10% underweight at the time of admission. Examining the same cohort, Ashley and Kannel (1974) reported that for each 10-unit increase in relative weight there was an increase of 6.6mmHg in systolic blood pressure in men and a more modest increase of 4.5mmHg in women.

Possible mechanisms of hypertension occurring in obesity include increased cardiac output (Alexander,1963), increased blood volume and sodium stores (Dustan,1983), abnormal aldosterone-renin response (Hiramatsu et al,1981), increased noradrenergic activity (Landsberg & Young,1978 and 1981a), and hyperinsulinaemia (Landsberg,1986).

1.2.3 c) Non-cardiovascular risks.

1) Digestive diseases

The association between obesity and gallbladder disease has been documented in several studies (Leijd, 1980). Increased cholesterol production and secretion provides one explanation for this association. Abnormalities in hepatic function occur commonly in obesity with steatosis occurring in 68-94% of obese patients (Braillon & Capron, 1983). Whether fibrosis or cirrhosis is present varies with the type of patient.

1i) Pulmonary disease and disability

Many abnormalities are seen on measurement of pulmonary function in obese persons (Luce, 1980). At one extreme are the patients with the Pickwickian syndrome (named after Joe, the fat boy in Dickens'

"Pickwick Papers"). The Pickwickian syndrome, or obesity hypoventilation syndrome, is characterised by somnolence, obesity and hypoventilation (Burwell et al, 1956). At the other extreme are patients with impairment in work capacity and pulmonary function due to obesity itself. A fairly uniform decrease in expiratory reserve volume (the volume of air that can be blown off after normal ventilation), a low maximum rate of voluntary ventilation and a tendency towards general reduction in lung volume occur in obesity. Respiratory muscles may also function abnormally in obese patients (Bray,1985).

iii) Endocrine disorders

A consistent reduction in the concentration of total serum testosterone is seen in obese men (Glass et al, 1981; Kley et al, 1980), that is directly related to the degree of obesity. A weight-related rise in both oestradiol and oestrone also occurs in men (Kley et al, 1980).

The onset of menarche frequently occurs at a younger age in obese girls than in normal weight girls (Bray,1976a). The obese patient often has irregular menstrual cycles as well as an increase in frequency of menstrual abnormalities including amenorrhoea, functional uterine bleeding, premature menopause and infertility (Rogers & Mitchell, 1952). Infertility is a common characteristic of the recessively inherited animal obesities including ob/ob, db/db and fa/fa mutants (Bray & York, 1971). In the dietary obese rat model used for these studies small differences between the cyclical variation in blood pressure and body weight change throughout the oestrous cycle were noted compared to lean rats (see section 3.3).

iv) Orthopaedic disorders

An increase in body weight might be expected to add additional trauma to the weight-carrying joints, thereby accelerating the development of osteoarthritis, an age-related, non-inflammatory disease of the diarthrodial joints characterised by degeneration and overgrowth of the cartilage and by overgrowth and sclerosis of the bone. Data on this issue are, however, contradictory. Surprisingly, in one study in which persons were more than 100% overweight, the incidence of osteoarthritis was only 12% (Saville & Dickson, 1968). However, several other studies have shown an increased prevalence of osteoarthritis in obese persons, as well as a greater mean weight for persons with osteoarthritis (Goldin et al, 1976; Weber, 1939). A study by Engel (1968) of the prevalence of osteoarthritis in hands (non-weight bearing) and ankles (weight-bearing) in a population of 2548 patients showed an increase in the prevalence of osteoarthritis in relation to body weight for all women over 35 years of age. The age-related increase in osteoarthritis was compensated for by dividing patients into four age groups. The increase in prevalence of osteoarthritis with increased weight was seen in all age groups.

v) Dermal abnormalities

Two cutaneous abnormalities have been associated with obesity. The first is fragilitas cutis inguinalis (Ganor & Even Paz, 1967). It was noted that in 63 out of 200 patients with a variety of diseases, apparently normal inguinal skin ruptured when stretched experimentally. This phenomenon was considered to be an expression of skin fragility in this region. The condition was related to obesity (nearly 70% of the patients showing this phenomenon were obese as compared to 20-25% who were of medium weight) but not to sex,

diagnosis or the presence of striae. Acanthosis nigricans, a darkening of the skin in the creases of the neck, in the axillary region and over the knuckles, is a second dermal abnormality associated with obesity. It is sometimes associated with highly malignant cancers, usually intra-abdominal adenocarcinoma occurring in middle-aged and elderly patients (Brown & Winkleman, 1968).

vi) Psychosocial disability

Studies of selected samples have shown that various kinds of psychosocial disability are more common in the obese. The obese are subject to social, economic and other discrimination (Cahrman, 1968). In Western societies, where slimness is projected as the ideal, obesity is a social stigma and possibly as a consequence, the obese are subject to self-disparagement and may have a poor self-image. However, there have been few large-scale population studies from which to draw firm conclusions and even fewer clinical trials assessing changes in psychosocial features.

vii) Cancer

A study by the American Cancer Society (Garfinkel, 1985) was the first large-scale prospective study to present data on overweight and cancer. It was reported that overweight men had significantly higher mortality ratios for colorectal and prostate cancer than normal weight men and that overweight women had much higher death rates for cancer of the endometrium, gall bladder and cervix and significantly higher rates for ovary and breast cancer than normal weight women. The overall mortality ratio for cancer for persons 40% or more overweight was 1.33 for men and 1.55 for women.

1.2.3 d) The role of weight reduction in the management of hypertension.

Benedict and Roth (1918) first discussed the potential role of weight control in the reduction of blood pressure. In a group of 25 normotensive subjects, they showed that a 10-20% weight loss produced a significant reduction in blood pressure in 56% of the subjects studied. In 15 out of 17 studies reviewed by Chiang et al (1969) a reduction in blood pressure after weight loss was demonstrated. These studies included normotensive or mildly hypertensive subjects.

One of the main reasons for failure to accept that weight loss has a causal role in the reduction of blood pressure has been the claim that the effect of caloric restriction on arterial pressure is mainly due to the concurrent restriction in sodium intake (Dahl et al, 1958). However, weight reduction has been reported in patients in whom sodium intake was maintained at 165 ± 52 mequiv/day in a diet in which calories were severely restricted; reduction in blood pressure (to normotensive levels in 75% patients) was reported and was maintained by more than half of the patients during a follow-up period of weight maintenance (Reisin & Frohlich, 1982). The effects of sodium on arterial blood pressure are discussed further in section 1.5.

Weight reduction has been shown to be superior to treatment with the beta-adrenoceptor blocking drug, metoprolol, in treatment of mild hypertension (MacMahon, 1985). Antihypertensive medication may produce unpleasant side-effects, which could impair the quality of life of the patient. For example, methyldopa, a widely-used antihypertensive drug worldwide, often causes sedation and dry mouth (Van Zwieten et al, 1984). Hydralazine, which acts directly on smooth

muscle to cause arteriolar vasodilation, is relatively free of central side-effects, however the main concern with this drug is that it is thought to cause drug-induced lupus syndrome (Kochwaser, 1976). Prazosin causes both venous and arteriolar vasodilation by its action as a postsynaptic (α_1) adrenoceptor blocker and is sometimes associated with orthostatic dizziness, lethargy, sexual dysfunction and nightmares (Veterans' Administration Co-operative Study Group, 1981). The drugs discussed so far are usually used for moderate and severe hypertension and so, in general, patients will accept the adverse effects in view of the long-term benefits of treatment. In such patients weight reduction would probably not totally abolish the need for medication, though it might reduce the dosage required for adequate control, thereby reducing the severity of dose-dependent side-effects (Wassertheil-Smoller, 1987). For mild hypertension the first line of treatment is usually with thiazide diuretics or beta-adrenoceptor blocking drugs. Thiazides are known to cause a number of adverse effects including hyperuricaemia, precipitation of diabetes mellitus and hyperlipidaemia (Maclean & Tudhope, 1983). Beta-adrenoceptor blockers may also cause a number of adverse effects. In addition to bradycardia, impairment of cardiac output and possible precipitation of asthma, they may cause tiredness of the legs, cold hands and feet and vivid dreams. Impairment of physical performance is perhaps the single most troublesome side-effect of this group of drugs in the young hypertensive patient (Lewis et al, 1984).

Hyperglycaemia, diabetes mellitus and certain hyperlipidaemias are all conditions closely associated with obesity (see section 1.2.3 b)). Thiazide diuretics, one of the most commonly used drugs for the

treatment of hypertension, have been shown to raise blood lipids and to predispose patients to diabetes mellitus (as stated earlier) and therefore their use in treatment of obese hypertensive patients might worsen the predisposition to these conditions. However, the use of weight reduction in the obese mildly hypertensive patient might be expected to improve the blood lipid profile and the hyperglycaemia whilst reducing the elevated blood pressure.

1.2.4 Methods of treatment of obesity

The connection between food intake in excess of energy expenditure and obesity was addressed by the famed French gourmet Brillat-Savarin in 1825:

"Any cure for obesity must begin with the following and absolute precepts: discretion in eating, moderation in sleeping, and exercise on foot or on horseback."

(Quoted by Kreutler, 1980)

In this section various methods used to treat obesity are discussed.

1.2.4 a) Dietary restriction

Fasting or starvation, in which no food is eaten, although water is taken, is the dietary measure which produces the greatest rate of weight loss. Fasting, as a therapeutic tool, has been known since the time of Hippocrates (translated by Adams, 1849). However, although it produces rapid weight loss it also carries a high risk of serious metabolic disorders and even death if continued for long periods due to the negative nitrogen balance occurring, with the loss of a large proportion of lean body mass in addition to loss of fat (Garrow, 1988).

Modified fasts, incorporating small amounts of high biological value animal protein to maintain positive nitrogen balance, were pioneered by Blackburn et al (1975) and Howard & Baird (1977). The early modified fasts gave rise to the many very low calorie diets (VLCDs) now marketed and available commercially, including the Cambridge Diet, the Microdiet (Uni-vite) and Modifast (Wander). VLCDs are usually presented as milkshake-type drinks or food bars containing high biological value protein, carbohydrate, fat and a vitamin and mineral premix, with a total energy content of approximately 330-500 calories in a days supply. VLCDs are very effective in producing rapid weight loss (Isaacs & Parry, 1984; Apfelbaum, 1987). The question of their safety can not be answered simply, since the safety depends on the duration of use and the health of the user. Garrow (1988) has presented a detailed review of many clinical studies involving the use of VLCDs.

By far the most popular and widely used type of diet for weight reduction is the low calorie diet, supplying approximately 1000-1500 calories per day, based on a selection of normal foods. There are three guiding principles in designing such diets. Firstly, the diet must supply less energy than the patient's maintenance requirements, otherwise there will be no weight loss. Secondly, it must supply all nutrients required apart from energy otherwise it will eventually lead to malnutrition. Thirdly, it must be (as far as possible) acceptable to the patient, otherwise the patient will not comply with the dietary advise. Low calorie diets offer many advantages over VLCDs and fasting: they may be tailored to fit the patients lifestyle and food preferences; as no special foods are needed, the patient may share meals with family and/or friends; they may include a varied,

balanced range of foods; they need not cost more than 'normal' meals; they may safely be continued until an appropriate weight is reached; they help to educate the patient about energy values of foods thus encouraging a change in long term eating habits and increasing the likelihood of weight maintenance once the excess weight is lost.

In the past there was a trend for weight-reducing diets to contain low levels of carbohydrate. So-called starchy foods were restricted and considered "fattening". However, recent research has provided evidence that it is foods with a high fat and/or a high refined carbohydrate content which should be restricted. Thus the most popular diets now tend to be based on foods with a low fat content which are also rich in complex carbohydrates (Hutton, 1985).

1.2.4 b) Behavioural modification

Interest in behavioural modification began in 1967 with the publication of a short paper, "Behavioral Control of Eating" (Stuart, 1967). This interest rapidly developed into an explosion of research.

A detailed account of a behavioural programme is beyond the limits of this section, however a brief description of some of the essential elements is as follows:

- i) self-monitoring - description of the behaviour to be controlled;
- ii) control of the stimuli that precede eating;
- iii) development of techniques to control the act of eating;
- iv) reinforcement of the prescribed behaviours
- v) cognitive restructuring.

Extensive descriptions of behavioural programmes have been

published by Mahoney & Mahoney (1976), Stuart (1978) and others. One particular large study has given grounds for optimism regarding the effectiveness of behaviour modification. Clinically significant weight losses (mean=10.9kg) were achieved in a large number of obese women, and these weight losses were maintained far more effectively than those achieved by drug therapy (Craighead et al, 1981).

1.2.4 c) The use of exercise in the treatment of obesity.

Increasing energy expenditure above energy intake is another way to achieve the negative energy balance required for weight loss. The components of energy expenditure by human beings can be divided into three parts: basal metabolism, heat losses due to the thermic effects of food and the energy needs for physical activity. The third component is the most easily manipulated and obviously depends upon the degree of activity. Increasing the level of physical activity will increase energy expenditure. Studies have shown that the use of exercise alone has produced less weight loss than other modalities (Wing & Jeffrey, 1979), however it is desirable to encourage patients to increase activity as an alternative to eating. Increased physical exercise is also useful in assisting the maintenance of a lower weight level after weight loss has been achieved.

Exercise may also prove useful in prevention of the decrease in metabolic rate seen with prolonged adherence to a low calorie diet. When dieting, metabolic rate may drop by 6% by the end of the first week and by 15-20% by the end of the third week (Hutton, 1985). Regular exercise might help to prevent the cycle of reduced rate of weight loss ('plateau effect') with subsequent abandonment of the diet, resulting in the lost weight being regained (plus possible

extra weight gain) and the metabolic rate remaining depressed. This process would leave the subject with a lower energy requirement and a greater likelihood of gaining weight in the future. There has been much interest recently in the theory that "dieting makes you fat", which is based on these observations (Cannon & Einzig, 1983).

1.2.4 d) Surgical treatment

Surgical treatment is generally reserved for morbidly obese patients, as defined by weight greater than 200% of ideal, though it may also be considered if the patient's weight is at least 300LB (135kg), or 100LB(45kg) in excess of ideal (Blackburn & Miller, 1983).

The various surgical procedures used are designed to induce weight loss by different means. The jejunoileal bypass (Payne et al, 1963; Payne & DeWind, 1969) is designed to shorten the intestinal tract and, by reducing intestinal absorptive capacity, to induce weight loss without the need for dietary therapy. The intention of gastric bypass and gastroplasty operations (Alden, 1977) is to reduce food intake by restricting stomach size.

Successful surgical treatment may be expected to produce 10-15LB (4.5-7kg) weight loss per month over a period of 9-15 months (Bothe et al, 1979). However, these techniques often produce medical complications, some of which may be severe and even life-threatening. The most common complications of the jejunoileal bypass procedures include pulmonary emboli, wound infection, renal failure and liver failure, with the latter being the most common cause of death in these patients (Bray et al, 1976). Other minor complications include diarrhoea, vomiting, minor electrolyte disturbances and

hypoproteinaemia. Complications of gastric bypass and gastroplasty are generally fewer in number and less major than those of jejunoileal bypass. However, the incidence of wound infection, dehiscence and pulmonary embolism are comparable for both procedures. The most serious complication of gastric bypass is an anastomotic leak (Blackburn & Miller, 1983).

Jaw-wiring or dental splinting, though not strictly a surgical technique, involves the splinting of the upper and lower jaws into a position such that only liquids or semi-solids may be ingested. It is basically an aid to "willpower" and its success depends upon adherence to a low calorie diet. Provided adequate dental hygiene procedures are followed, complications of this technique are few, though there may be considerable pain for the first few days after wiring. The resulting weight loss may be considerable, though much weight is often regained once the wires are removed. In order to try to avoid this rebound weight gain, Garrow and Gardiner (1981) adopted a treatment policy of jaw-wiring plus a waistcord. A 2mm diameter cord was applied when the wires were removed. Patients who used it reported that the discomfort of the tight waistcord was a cue to restrict food intake until it loosened again. From the results of the study Garrow and Gardiner (1981) concluded that the waistcord was a valuable aid to successful and permanent weight loss in some subjects.

1.2.4 e) Pharmacotherapy of obesity

In theory a drug may effect weight loss in one or more of the following ways: by reducing food intake (category A); by decreasing food absorption (category B); by increasing energy expenditure

(category C).

The first chemical to be marketed specifically as an anti-obesity drug fell into category C. Dinitrophenol increased basal metabolic rate and energy expenditure by inhibition of energy-rich phosphate bond transfer. Unfortunately, this agent was found to produce wide ranging toxicity, including cataracts, and was therefore withdrawn (Lasagna, 1983). Thyroid preparations (category C) were used in the past for treatment of obesity, in part because of the fallacious belief that obese patients were hypothyroid. However, potentially dangerous side-effects such as cardiac arrhythmias have led to their use in obesity being discouraged. However, Gibbs (1988) recently reported the occurrence of ventricular fibrillation in a woman who was taking thyroid (USP) in combination with an anorectic drug and a diuretic for treatment of moderate obesity. This report demonstrates that such drugs are still being used inappropriately for the treatment of obesity with potentially hazardous results.

A group of compounds known as "starch blockers" (category B), which were said to inhibit the action of pancreatic amylase and thus prevent the digestion and absorption of starchy food, raised considerable interest in the early 1980's (Hanssen, 1982). However these compounds proved to be ineffective in producing weight loss.

All of the drugs currently marketed in this country for the treatment of obesity fall into the class of appetite suppressants or anorectic drugs (category A). The amphetamines were the first drugs of this class. However, the undesirable cardiovascular and central nervous system stimulation and also the abuse potential of these drugs has resulted in their use as anorectic agents being discouraged. (See section 1.4.2 for further details of the uses and

actions of amphetamine). Newer anorectic drugs, many of them based on the structure of amphetamine, were designed to retain the anorectic activity of amphetamine without the unwanted side-effects. Diethylpropion, phentermine, fenfluramine and mazindol are the anorectic agents currently available for general clinical use in the United Kingdom. Diethylpropion, phentermine and mazindol have stimulant properties whereas fenfluramine tends to produce sedation. Mazindol is the only current anorectic drug structurally unrelated to amphetamine (see sections 1.4.3 and 1.4.4 for details of the pharmacology of fenfluramine and mazindol).

Bulking agents such as methylcellulose are listed in the British National Formulary as appetite suppressants, though it states that there is little evidence to support their efficacy in producing satiety at the doses present in the available preparations. Ray et al (1983) were able to demonstrate a delay in gastric emptying time with treatment with methylcellulose, though no weight loss occurred.

Thermogenic drugs (category C) are the subject of much current research. These drugs, which include BRL26830A, R016-8714 and LY104119, appear to stimulate lipolysis and energy expenditure by a direct stimulation of beta-adrenoceptors (Arch et al, 1984b); Meier et al, 1984; Yen et al, 1984). Chronic administration of these drugs has produced promising effects of weight loss in obese rodents (Arch et al 1984a) & 1984b)), though the results of clinical studies in obese humans have not all shown weight loss. Chapman et al (1985) showed no significant weight loss in refractory obese patients with treatment with BRL26830A compared with placebo. However, a recent study (Connacher et al, 1988) showed a greater weight loss in patients who received BRL26830A (15.4kg, SD6.6) than in those who

received placebo (10.0kg, SD5.9). The subjects received either placebo or BRL26830A daily for 18 weeks and were instructed to follow a low fat/ high fibre low calorie diet. Measurements of skinfold thickness indicated that the weight loss with BRL26830A was mainly from adipose tissue and not from lean tissue. From these results it was concluded that BRL26830A might be a useful drug, as an adjunct to dietary therapy, in the treatment of obesity.

1.2.5 Animal models of obesity

Although animal obesities can not represent a complete model of the human condition, they have provided important insights into the causes and consequences of the disease as well as providing a useful way of assessing potential treatments. They may be classified under the following headings.

a) Neural models

Among the neural models hypothalamic obesity is the most well known. Originally produced by electrolytic lesions of the ventromedial hypothalamus, (VMH), (Hetherington & Ransom, 1940), there are now several ways of inducing hypothalamic obesity, including knife-cuts through the medial hypothalamus, lesions of the paraventricular hypothalamus and systemic injections of gold thioglucose, monosodium glutamate or bipiperidyl mustard (Sclafani, 1984). Bilateral hypothalamic damage is usually necessary to produce major obesity.

b) Endocrine models

The most well-characterised of the endocrine-related obesity syndromes is that produced by ovariectomy (Wade & Gray, 1979). Chronic administration of insulin (Lotter & Woods, 1977) and

glucocorticoids (Hollifield, 1968) have also been used to increase food intake and body weight.

c) Pharmacological treatment

No pharmacological model of obesity has yet been fully developed. 5-hydroxytryptamine antagonists such as cyproheptadine have been shown to produce weight gain in rats (Oomura et al, 1973). Clonidine has been shown to produce hyperphagia and weight gain in monkeys (Schlemmer et al, 1979). Chlordiazepoxide has been shown to increase food intake and body weight in the rat (File, 1980). Surprisingly, a single large dose of amphetamine has been shown to induce a mild, long-lasting obesity in rats (Hoebel et al, 1981).

d) Nutritional obesity

Experimental obesity can be produced by a variety of nutritional manipulations. The most direct approach to inducing additional weight gain is to force-feed excess calories and this technique has been used to produce primate models of obesity. Increasing the palatability of the diet (high-fat diets, high-sugar diets and supermarket/cafeteria diets) has proved to be an easy and effective means of inducing obesity in rats (Sclafani, 1978). The cafeteria or supermarket diet, providing an assortment of highly palatable, calorically-rich foods was used to produce the dietary obesity in the rats used in the studies described here. The varied palatable diet used was based upon the sort of foods used by Sclafani and Springer (1976). This model was considered the most appropriate for the studies because it seemed to be the most representative mirror of the human condition of simple obesity. At present, the aetiology of most human obesity is unknown, however there is usually no evidence of endocrine imbalance, neural disorder, pharmacological cause or

metabolic dysfunction. The rats offered the palatable diet appear to become obese because they overeat the foods which they 'enjoy'. The restrictive housing provided by the cages, limiting the amount of exercise possible, may contribute to the development of obesity (see section 1.2.5 e)). Similarly, lack of exercise and overeating are often cited as causes of simple obesity in man. The dietary obese rat also exhibits some of the conditions associated with human obesity, including mildly elevated blood pressure (Kirby, 1984).

e) Environmental factors

The limited opportunity for physical activity provided by normal laboratory housing is a factor in most, if not all, obesity models. This is indicated by the fact that voluntary or forced running activity suppresses body weight and/or body fat gain in hypothalamic, dietary and genetic obesity models (Sclafani & Rendel, 1978).

A more unusual environmental manipulation which has been reported to promote overeating and obesity in the rat is tail-pinching (Rowland & Antelman, 1976). This has been proposed as a model of "stress-induced" eating (see section 1.2.2 e) ii)).

f) Genetic obesities

Models in this class have been divided according to the mode of inheritance: single-gene dominant strains, single-gene recessive strains and the polygenic inbred or hybrid strains. Of these the single-gene recessive models, in particular the obese mouse (ob/ob), the diabetic mouse (db/db) and fatty Zucker rat (fa/fa) have been the most extensively studied. These genetically obese animals all have severe metabolic derangements (Sclafani, 1984). The ob/ob and db/db mice exhibit severe hyperglycaemia and hyperinsulinaemia, whilst the fa/fa rat shows hyperlipidaemia. These metabolic changes are more

severe and occur to a greater extent in these animal models than is known to be the case in the human condition of obesity.

g) Miscellaneous

A novel form of obesity is that produced by a viral infection (Lyons et al, 1982). Mice infected with canine distemper virus have been found to significantly increase their body weight as well as their fat cell size and number, compared to control mice. The obesity was associated with reduced brain catecholamine levels and therefore may represent another form of neurally-mediated obesity. Another unusual form of obesity is that which spontaneously appears in individual laboratory animals for no known reason (Sclafani et al, 1980).

In addition to the models already described, it is also possible to produce mixed models of obesity. For example, additive effects on body weight can be produced by combining, in the same animals, VMH and midbrain lesions or medial hypothalamic knife cuts and ovariectomy (Gale & Sclafani, 1977). VMH lesions and medial hypothalamic knife cuts also potentiate the obesity produced by palatable diets (Bray & York, 1979).

1.3 THEORIES OF FEEDING BEHAVIOUR

1.3.1 Anatomical location of feeding control sites

Many workers have conducted experiments to locate the anatomical site(s) of feeding regulation in the brain. Early studies by Hetherington and Ransom (1940), using rats, showed that destruction of the ventromedial hypothalamus (VMH) produced hyperphagia and obesity. A few years later Anand and Brobeck (1951) showed that

damage of the lateral hypothalamus (LH) caused both rats and cats to stop eating. These effects in the VMH and LH suggested that the hypothalamus contained primary control areas for both hunger and satiety and resulted in a two-centre theory of regulation of food intake with the VMH being referred to as a "satiety centre" and the LH as a "feeding centre" being proposed by Stellar (1954). Soon problems with the available evidence for the two-centre hypothesis of control of food intake appeared. For example, lesions of the lateral hypothalamus, which produced aphagia, might also damage fibre pathways coursing nearby, such as the dopaminergic nigrostriatal bundle, and damage to these pathways outside the lateral hypothalamus could produce aphagia (Marshall et al, 1974). It became evident that the effects of lesions to the VMH and LH are reproducible with lesions to the ventral noradrenergic bundle and the dopaminergic nigrostriatal system (Ahlskog & Hoebel, 1973; Ungerstedt, 1971). It was also discovered that the two 'centres' consisted largely of fibres of passage, noradrenergic in the VMH and dopaminergic in the LH (Ungerstedt, 1971), thus at this time it was unclear what, if any, role the hypothalamus played in the control of feeding. To obtain more direct evidence on the neural processing involved in feeding, the activity of single neurones in the hypothalamus and other brain regions such as the amygdala, prefrontal cortex and striatum was analysed (Rolls, 1985). It was found that a population of neurones in the lateral hypothalamus and substantia innominata of the monkey responded to the taste and/or sight of food by increasing their rate of firing. This effect was only seen when the animals were food-deprived and the cells no longer responded when the animals were replete. Thus, it appears that the hypothalamus is an area where the

response to external food-related cues is integrated with the animals inner state of food deprivation or repletion. Such a centre would monitor metabolic status. This could be via glucose receptors either within the hypothalamus itself as proposed by Oomura (1976) or in the liver as suggested by Russek (1981). It has been shown that local application of glucose directly onto the VMH and LH, where glucose receptors and glucose-sensitive neurones have been demonstrated, (Oomura et al, 1964), induced increased neuronal firing in the VMH and reciprocally decreased LH neuronal activity (Oomura et al, 1967). Free fatty acids (Oomura, 1976) and amino acids (Panksepp, 1975) have also been shown to have effects on neuronal discharge frequency and food intake respectively, when administered locally to the VMH and LH.

The stimulatory effect upon LH neurones of external food-related cues, which require modification by learning, implies that forebrain areas such as the inferior temporal visual cortex and the amygdala are also involved in the control of feeding behaviour. This conclusion is consistent with the anatomy of the hypothalamus and substantia innominata, which receive projections from limbic structures such as the amygdala, which in turn receive projections from the association cortex. The conclusion is also consistent with the evidence that decerebrate rats retain simple controls of feeding, but do not show normal learning about foods (Grill & Norgren, 1978).

The importance of visual inputs and learning to feeding in relation to which some hypothalamic neurones respond, is that animals and especially primates, may eat many foods every day and must be able to select foods from other stimuli, as well as produce the appropriate preparative responses such as salivation and the release of insulin.

In addition to the central control of feeding, possible peripheral contribution must also be considered. Many types of negative feedback signals, which limit food intake, have been identified (Davis et al, 1976). These include osmotic signals, gastric distension, hepatic glucoreceptor activity (Russek, 1981), duodenal hormones, chemospecific activity and tension receptor activity in the wall of the small intestine.

1.3.2 Chemical transmitters involved in the regulation of feeding

Within the hypothalamus of laboratory animals, the catecholamines noradrenaline (NA) and dopamine (DA) together with 5-hydroxytryptamine (5-HT, serotonin) have been shown to be involved in the regulation of feeding behaviour (Leibowitz, 1982; Morley & Levine, 1983; Hoebel, 1984; Blundell, 1985). In human subjects there is evidence from drug studies that some of these same neurotransmitter substances are involved in the experience of hunger and feeding. Amphetamine, a drug which promotes the release of NA and DA, profoundly reduces hunger and diminishes food intake (Silverstone & Stunkard, 1968). This effect can be attenuated, at least in some subjects, by the NA receptor-blocking drug thymoxamine, suggesting that NA may be involved in human feeding, as it is with laboratory animal feeding (Silverstone & Goodall, 1984). The pharmacological evidence for the role of 5-HT in human feeding is derived from three types of investigation. Firstly, the drug fenfluramine, which has been shown to act on the serotonergic system by the release of 5-HT from nerve endings and the inhibition of its re-uptake into the neurone (Garattini & Samanin, 1976; Garattini, Buczko, Jori & Samanin, 1975) has been shown to have anorectic activity (see also section 1.4.3 for

details of the pharmacology of fenfluramine). However, Dourish et al (1985) observed that the 5-HT_{1A} agonist 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) induced hyperphagia in non-deprived rats. This finding was unexpected, as previous studies had suggested that 5-HT agonists produced anorexia. Further investigations (Dourish et al, 1986) showed that the effect of 8-OH-DPAT could be abolished by pretreatment with the 5-HT depleting agent p-chlorophenylalanine (PCPA), and that direct infusion of 8-OH-DPAT onto the raphe nuclei, where somatodendritic 5-HT_{1A} binding sites have been identified, elicited hyperphagia, (Hutson et al, 1986). These results indicate that 8-OH-DPAT causes hyperphagia by stimulating 5-HT_{1A} somatodendritic autoreceptors and decreasing 5-HT function. The putative 5-HT_{1B} agonist RU24969 induces anorexia, which is not abolished by pretreatment with PCPA (Kennett et al, 1987), suggesting that 5-HT_{1B} agonists induce anorexia at a postsynaptic 5-HT receptor.

Secondly, additional evidence for the role of 5-HT in the control of feeding comes from the observation that dietary tryptophan, the amino acid precursor of 5-HT which has been shown to influence the levels of 5-HT in the rat brain (Fernstrom, 1981), has been shown to cause a dose-related suppression of total calorie intake in healthy human volunteers (Silverstone & Goodall, 1984).

Thirdly, cyproheptadine, an antihistaminic compound with potent antiserotonergic properties, has been shown to increase hunger rating, food intake and weight gain in normal subjects (Silverstone & Schuyler, 1975).

Other transmitters which may be involved in the regulation of feeding include GABA (gamma amino butyric acid), opioid peptides, CCK (cholecystokinin) and bombesin. When injected directly into the VMH

of rats, GABA increased food intake but in the LH it gave variable effects, while it suppressed feeding when applied to the dopaminergic neurones of the substantia nigra (Kelly et al, 1977). The GABA antagonist bicuculline methiodide, when injected directly into the LH, increased ingestion of sweetened milk in rats (Kelly et al, 1977), suggesting that GABA may inhibit feeding in the LH.

There is substantial evidence for the role of endogenous opioid peptides in the regulation of feeding (Morley et al, 1984). In the rat activation of both the dynorphin-kappa opioid receptor (Morley & Levine, 1981) and the beta-endorphin-epsilon opioid receptor (Morley & Levine, 1982a) appear to enhance feeding, most probably acting in different areas of the central nervous system. It also appears that rats may have a mu anorectic system (Morley et al, 1982b; Sanger & McCarthy, 1980). Too few studies have been undertaken to define whether the delta or sigma receptor systems are also involved in feeding responses. It has become apparent that a great deal of species diversity exists in the feeding responses to opiates, making it difficult to extrapolate results obtained in rats to other species. In humans, studies with naloxone suggest an opioid-sensitive feeding system, which is possibly specifically involved in the regulation of carbohydrate intake (Morley & Levine, 1982a; Morley et al, 1984). Spiegel et al (1987) showed a significant reduction in meal size with naltrexone during a study in which the dose was increased from 25-200mg/day over 4 days. However, Malcolm et al (1985) found no significant effect on weight loss with 8 weeks treatment with naltrexone 200mg/day. Butorphanol tartrate (20µg/kg) was found to decrease feeding in humans and this effect was partly reversed by naloxone at a dose (6µg/kg) which would only be expected

to antagonise mu receptors (Morley et al, 1984). These data suggest that in humans there may exist a mu anorectic system.

CCK or the synthetic octapeptide CCK-8 have been shown to inhibit food intake in rats without producing malaise (Gibbs et al, 1973). It has been suggested that CCK-8 may inhibit food intake by altering the orosensory or hedonic influence over feeding in rats (Waldbillig & O'Callaghan, 1980; Bartness & Waldbillig, 1984). A study in human volunteers by Sturdevant & Goetz (1976) showed that CCK may produce either stimulation or inhibition of food intake, depending on the dose and duration of administration.

Bombesin (by intraperitoneal injection) has been shown to suppress food intake in the rat in a dose-dependent manner. It shortened the duration of the meal but did not delay or prevent onset of eating, thus it was suggested as a putative satiety signal (Gibbs et al, 1979).

There is evidence that food composition may have a direct effect on brain amine levels and a feedback control system is thought to exist (Wurtman & Wurtman, 1986; Wurtman, 1987). Wurtman proposed that a carbohydrate-rich meal has the effect of reducing plasma concentrations of most large neutral amino acids (LNAA) with the exception of tryptophan (TRP). Therefore the ratio of TRP to other LNAA in the plasma is increased. TRP and the other LNAA share a common transport system across the blood-brain barrier and therefore a relative increase in the concentration of TRP in plasma will facilitate the transport of TRP into the brain. TRP is a precursor of 5-HT and the rate of entry of TRP into the brain is the rate-limiting step in brain 5-HT synthesis. Therefore an increase in the concentration of TRP (and consequently of 5-HT) in the brain would be

expected to occur. A protein-rich meal has the opposite effect on the ratio of plasma TRP to other LNAA. It has been observed that prior ingestion of carbohydrate reduces subsequent carbohydrate intake in rats (Wurtman et al, 1983), thus maintaining a fairly constant ratio of carbohydrate to protein in the diet (Theall et al, 1984). Similar mechanisms may operate to govern macronutrient choice in humans, as it appears that the protein-carbohydrate ratio of food intake varies only within a very narrow range from day to day (Wurtman et al, 1981; Wurtman et al, 1985). Thus it has been proposed that serotonergic drugs such as fenfluramine may act by "fooling the brain" into thinking a high carbohydrate meal has been eaten, such that a reduction in carbohydrate intake at the next meal will result (see section 1.4.3 for details of the pharmacology of fenfluramine).

1.3.3 Mediators of energy balance

The brain is not a storage site for fat, protein or carbohydrate. This suggests that it regulates the amount of energy in the body by monitoring the circulating level of some mediator(s) between the brain and energy storage sites. Such a mediator should be shown to vary with the nutritional status of the individual. Secondly, manipulations of the level of the mediator, either directly in the brain or in the circulation, should induce changes in feeding and energy expenditure. A number of such substances have been suggested as mediators of energy balance.

a) Glucose and the glucostatic theory

The glucostatic theory of energy regulation, first postulated by Mayer (1953), considered glucose as the mediator of energy balance. According to this theory, there are glucoreceptors with special

affinity for glucose in the VMH and their utilisation of glucose varies directly with the level of glucose in the circulation. Increased utilisation by the glucoreceptors in the VMH would activate mechanisms which inhibit feeding, while decreased utilisation produces the opposite effect on feeding. The existence of glucoreceptors and glucosensitive neurones in both the VMH and LH of rats has been demonstrated (Oomura et al, 1964). Duodenal and hepatic portal infusions of glucose have been found to be effective in decreasing food intake in hungry rats (Novin et al, 1974) and experimentally-induced cellular glucoprivation using the glucose analogue 2-deoxy-D-glucose has been reported to result in increased food intake in rats and monkeys (Smith & Epstein, 1969). Direct implants of gold-thioglucose (GTG) another glucoprivation agent, in the presence of insulin, induces hyperphagia and obesity in rats (Smith & Britt, 1971).

b) Lipids and the lipostatic theory

Kennedy postulated the lipostatic theory for energy balance in 1953. He proposed that information reflecting the size of the body's fat stores is conveyed by metabolites from the fat stores to the brain. The brain would then maintain body weight at a 'set point' on the basis of this information. Displacement of body weight beyond the 'set point' would induce changes in the intensity of the signal (metabolite) to the brain which could evoke alterations in the processes in the regulatory mechanism to restore normal body weight. The mechanism for this could be the rate of lipolysis and triglyceride synthesis by re-esterification of locally available free fatty acids (FFA) or esterification of FFA from other sources which have been found to be directly proportional to the size of adipocytes

and hence to the size of fat depots (Smith, 1970; Otto et al, 1976). A variety of metabolites from adipose tissue have been proposed as candidates for the peripheral signal. Walker and Remley (1970) proposed FFA. Another candidate is glycerol. Many investigators have observed reductions in food intake induced by either intra-peritoneal or subcutaneous administration of glycerol (Racotta & Russek, 1977). Wirtshafter and Davis (1977) in their experiments involving long-term administration of glycerol in rats, produced effects similar to those observed after LH lesions. They suggested that the effect of long-term treatment with glycerol was re-setting the body weight 'set point' at a lower level.

c) Amino acids and the aminostatic theory

The dependence of food intake in experimental animals on the quantity of proteins and the quality of protein, that is, the amino acid balance of the protein, in the diet (Harper, 1976) has led to speculations that amino acids also play a role in the control of food intake and hence in energy balance.

Harper (1976) showed that a diet deficient in histidine induced a spontaneous decrease in food intake in rats. However it has been found that the suppression of food intake induced by manipulating dietary protein content is transitory (Anderson et al, 1968), showing adaptation to alterations within a few days. The occurrence of adaptation, leading to restoration of normal levels of food (and energy) intake, suggests that the responsiveness to alterations in the protein content of the diet is not a basic component of regulation of food intake and energy balance, but might be a component of a fine-tuning mechanism. The effect of dietary tryptophan on food intake has been discussed in section 1.3.2.

d) The thermostatic theory

The thermostatic theory for the regulation of energy balance in animals was proposed by Brobeck (1948) on the basis of results of studies to investigate the dependence of food intake on environmental temperature. The theory postulated that food intake is regulated by heat production, as reflected by changes in body temperature. Food intake in rats has been shown to be dependent upon environmental temperature conditions (Hamilton, 1963). A cold environment (8°C) increasing and a hot environment (35°C) decreasing food intake relative to the normal intake at 24°C .

It is known that overnutrition in the rat results in brown adipose tissue hyperplasia and increased thermogenesis (see section 1.2.2). The thermostatic theory proposes that the increased thermogenesis would in turn cause a reduction in food intake.

1.4 REVIEW OF THE ANORECTIC DRUGS USED

1.4.1 General introduction

Three anorectic drugs were chosen for these studies; amphetamine(d), fenfluramine(dl), and mazindol. Amphetamine was used because it is the classic anorectic agent against which other newer compounds are frequently compared. It is thought to act predominantly by dopaminergic and possibly noradrenergic pathways (see below). Fenfluramine was selected because it is widely used clinically in the treatment of obesity in the U.K., and unlike most other anorectic agents it is non-stimulant. It appears to act via serotonergic mechanisms (see below). Mazindol was selected as a stimulant anorectic agent which is used clinically. It is, like amphetamine,

thought to act via dopaminergic pathways (see below). It is structurally unrelated to the phenylethylamine anorectic agents (see Fig.1)

1.4.2 Amphetamine

Amphetamine was first synthesized in 1887 by Edeleano, but no pharmacological studies were reported until 1910 when Barger and Dale described some of the sympathomimetic properties. Its pressor effects and dryness of mouth (sympathomimetic effects) were noted in man by Pinness et al (1930). The central stimulant effects were first used clinically by Prinzmetal and Bloomberg (1935) to treat narcolepsy.

One of the first reports of the efficacy of amphetamine in the treatment of obesity was by Lesses and Myerson (1938), however, subsequent studies with obese patients showed a reduced rate of weight loss due to tolerance (Aldesburg & Meyer, 1949). The problem of central stimulation as a side-effect was also noted in this study. Therefore, although amphetamine is regarded as the prototype anorectic agent against which other potential anorectic drugs are evaluated it is rarely used clinically for the treatment of obesity, due to the central nervous system stimulant effects and the abuse potential. Also its safety, bearing in mind its cardiovascular stimulant action, is questionable and it was reported in a recent study (Carson et al, 1987) to have been contributory to myocardial infarction in drug abusers. A working party of the British Medical Association in 1968 recommended that amphetamine and the amphetamine-like compounds should only be used where there was no alternative treatment and should be avoided in the treatment of obesity.

Amphetamine has been shown to cause a release of catecholamines (noradrenaline and dopamine) from nerve terminals in the brain and to block catecholamine re-uptake (Carlsson, 1970). There is considerable evidence that amphetamine exerts its anorectic action primarily by interaction with catecholamine-containing neurones in the brain (see section 1.3.2, Theories of Feeding Behaviour). Studies have shown that selective electrolytic lesions placed in the ventral noradrenergic bundle of the rat markedly diminished the anorectic action of amphetamine (Ahlskog, 1974). Also, pre-treatment of rats with desipramine to protect noradrenergic terminals from the neurotoxin, 6-hydroxydopamine, prevented the attenuation of anorectic potency of amphetamine by the neurotoxin (Garattini et al, 1978). These observations suggest the involvement of noradrenergic systems in the amphetamine-induced suppression of feeding.

In addition to interactions with central neurotransmitters, amphetamine also induces alterations in peripheral metabolism of lipids. It has been reported that acute doses of amphetamine increase plasma free fatty acid levels in fed animals (Fassina, 1964 & 1966; Von Herold et al, 1965) and in man (Pinter & Pattee, 1968). However, the mode of action of amphetamine in causing weight loss appears to be almost entirely a centrally-mediated reduction in food intake, with only a small effect on metabolic rate (Harris et al, 1947). In the rat and in man tolerance rapidly develops to the anorectic effect of amphetamine (Bowman & Rand, 1980). In double-blind human studies amphetamine has been shown to produce significant suppression of appetite, hunger rating and body weight (De Felice et al, 1973), while in rats microstructural analysis of feeding behaviour showed that amphetamine markedly reduced the latency to the initiation of

eating and actually caused rats to markedly increase the rate of consumption, whilst decreasing both size and duration of each eating bout (Blundell & Latham, 1978). Thus again suggesting the drug probably acts by suppressing hunger.

In developing newer anorectic drugs, the aim has been to retain the anorectic properties of amphetamine whilst abolishing the central stimulant effects, the development of tolerance and the cardiovascular side-effects, of which the most important is an increase in blood pressure due to both increased heart rate and increased peripheral vascular tone (Kalant, 1975).

1.4.3 Fenfluramine

Fenfluramine is an anti-obesity agent structurally related to amphetamine (see Fig.1) whose anorectic activity has been well established in animal studies (Le Douarec et al 1966; Alphin & Ward, 1969). Its efficacy in the treatment of human obesity has also been well documented (Duncan et al, 1963; Munro et al, 1966; Silverstone et al, 1970). Unlike amphetamine, fenfluramine does not produce central stimulation, in fact it tends to be slightly sedative (Craddock, 1978). It has been suggested that the food intake suppressant effect of fenfluramine is primarily mediated by central serotonergic mechanisms (Samanin, 1983), see section 1.3.2. Evidence for this theory includes studies which show that the anorectic effects of fenfluramine are abolished by 5-HT antagonists such as methergoline (Jespersen & Scheel-Kruger, 1973) or methysergide (Blundell & Lesham, 1973). Lesions of the midbrain raphe nuclei, which selectively deplete 5-HT, also abolish the effect of fenfluramine (Samanin et al, 1972). A study by Wong (1987) proposed
& Reid

involvement of the 5-HT_{1A} subtype receptor in the control of feeding, having shown that fenfluramine antagonises the stimulation of food intake induced by the putative 5-HT_{1A} agonist, isapirone in non-fasted rats.

There is evidence suggesting that fenfluramine may also owe some of its anti-obesity action to metabolic effects which aid the utilisation of energy substrates. It has been shown, like amphetamine, to cause an increase in levels of free fatty acids, glycerol and ketone bodies in humans, suggesting enhancement of fat mobilization and catabolism by the drug (Pawan, 1969). Bizzi, Venoroni and Garattini (1973) showed a decreased absorption of triglyceride after an olive oil load in rats following treatment with fenfluramine. This inhibitory action on intestinal absorption of triglycerides may partly explain the anti-hypertriglyceridaemic effect of fenfluramine and has in turn been attributed to inhibition of pancreatic lipase (Danneburg & Ward, 1971), monoacyl glyceryl acyltransferase (Danneburg et al, 1973) and intestinal motility (Bizzi et al, 1973).

The reduction in intake of a glucose solution by rhesus monkeys, produced by fenfluramine (2mg/kg intragastric) has been shown to be strongly related to the degree of inhibition of gastric emptying observed (Robinson et al, 1986). Thus, the results support an important role for the inhibition of gastric emptying in fenfluramine-induced suppression of feeding.

The first work suggesting that fenfluramine might alter glucose metabolism came from the study by von Herold et al (1965), who showed lowered blood glucose levels in rats treated with fenfluramine. The ability of fenfluramine to increase glucose utilisation was

demonstrated 'in vivo' by Butterfield and Whichelow (1968) using the forearm perfusion technique in volunteers. Kirby and Turner (1975) showed that fenfluramine increased glucose uptake into rat isolated skeletal muscle, the effect being insulin-dependent and dose-related. This response was antagonised by methysergide. The hypoglycaemic effect of fenfluramine makes it particularly suitable for the treatment of the obese non-insulin-dependent diabetic.

Analysis of the microstructure of feeding in rats revealed that fenfluramine reduced the rate of eating and induced premature termination of meals, suggesting that the drug probably enhances satiety (Blundell et al 1979). In animals given high-protein and low-protein diets it has been shown that fenfluramine decreases consumption of low-protein/high-carbohydrate diet and total food intake whilst sparing protein consumption (Wurtman & Wurtman, 1977). This protein-sparing reduction in food intake has also been shown in human subjects (Wurtman & Wurtman, 1981). The theory proposed to explain the protein-sparing effect of fenfluramine postulates that the ability of fenfluramine to suppress carbohydrate intake selectively and to sustain protein intake probably relates to the similarity between its neurochemical effects and those of dietary carbohydrate(see section 1.3.2) It is proposed that the enhancement in serotonergic transmission that follows administration of fenfluramine conveys to the brain the false signal that carbohydrate has recently been consumed. As stated in section 1.3.2, both animals and man tend to regulate the proportion of protein in the diet and therefore this false signal of prior carbohydrate consumption would be expected to decrease subsequent carbohydrate appetite. However, evidence for this theory is not conclusive and other workers have not

always been able to show selective suppression of carbohydrate intake with fenfluramine or other serotonergic agents such as fluoxetine and MK212 (Curtis-Prior & Prouteau, 1983).

1.4.4 Mazindol

Mazindol was first synthesized in 1966 and was the first drug marketed for the treatment of obesity which is not structurally related to amphetamine, being an imidazoisindole (see Fig.1).

Although it is known that mazindol alters catecholamine metabolism (Carruba et al, 1978), its mechanism of action has not been fully elucidated. It has been observed that anorectic doses of mazindol (4mg/kg) block the uptake of ³H-noradrenaline in rat brain by 80% (Engstrom et al, 1975). On the basis of this observation, Gogerty (1981) suggested that the anorectic effect of mazindol is determined by its inhibition of noradrenaline uptake in the brain. However, attenuation of the anorectic effect of mazindol by pretreatment of rats with pimozide (a dopamine receptor blocker) suggests a role for dopaminergic mechanisms in the anorectic action of mazindol (Kruk & Zarrindast, 1976). They found no effect on mazindol-induced suppression of food intake when animals were pre-treated with phenoxybenzamine (alpha-adrenoceptor blocker), propranolol (non-selective beta-blocker) or methergoline (5-HT receptor blocker).

In obese diabetics and obese women, it has been shown that mazindol administration results in increased plasma concentrations of free fatty acids and decreased concentrations of cholesterol and triglycerides (Rath et al, 1979). A non-insulin-dependent enhancement of glucose uptake into human isolated skeletal muscle induced by

mazindol was reported by Kirby and Turner (1976). Chronic administration of mazindol has been reported as having a hyperinsulinaemic effect (Sirtori et al, 1971), in acute studies Harrison et al(1975) observed reduced levels of immunoreactive insulin, and Rath et al (1979) found no significant effect of mazindol on plasma insulin levels in obese women. Mazindol has been shown to increase resting metabolic rate in rats (Wyllie et al, 1984) and to prevent the fall in energy expenditure normally associated with a reduction in food intake.

In double-blind clinical trials Smith et al (1975) reported that mazindol induced significant reduction in body weight when compared to placebo treatment. As an adjunct to dietary and behavioural treatments, it has been shown that mazindol causes an increased mean weight loss in both cases (Wallace, 1976).

1.5 SODIUM, POTASSIUM AND OTHER DIETARY FACTORS IN HYPERTENSION

As mentioned briefly in section 1.2.3d), it was proposed by Dahl et al (1958) that the lowering of blood pressure associated with weight loss is due wholly or mainly to the reduction in sodium or salt intake which tends to occur when food intake is restricted. Dahl's theory proposed that a high dietary sodium intake was associated with high levels of blood pressure. Evidence for the role of sodium and also potassium in the control of blood pressure and the aetiology of hypertension are discussed here.

1.5.1 Epidemiological studies

There have been three major studies in the Pacific region in which different sodium intake has been implicated as a factor

responsible, in part, for variations in mean blood pressure levels and the prevalence of hypertension among populations. Early in the 1960's Prior et al investigated blood pressure and salt intake among the Polynesian population of the Cook Islands (1968; 1969). The inhabitants of Pukapuka (an outlying atoll of the northern Cook group) had lower blood pressures, were slimmer and had a lower salt intake than the modernized population of Rarotonga, the main island of the group. The differences in blood pressure were greater than those accounted for by height and weight, and it was concluded that the findings were compatible with Dahl's hypothesis that "higher salt intake and higher blood pressures are related".

An investigation of the antecedents of cardiovascular disease in six societies in the Solomon Islands late in the 1960's showed that both blood pressure and salt intake generally increased with acculturation (Page et al, 1974). An exception was a fishing community in which sea-water was used for cooking; this population had the highest mean salt intake and mean blood pressure, although the society ranked in the middle of the acculturation scale. The conclusion again was that the findings were compatible with Dahl's hypothesis.

A study of cardiovascular disease in Fiji in 1980 documented higher mean blood pressure samples in urban populations studied compared with the rural population sample. The rural subjects were leaner and had lower sodium concentrations in their early-morning urine samples (Ram et al, 1982). There have been several studies in other parts of the world which have also documented the association of a low salt intake with low levels of blood pressure, which may fail to rise with age (Oliver et al, 1975) and the association of

higher blood pressure with higher salt intake (Sasaki, 1964). However, there have been studies in which this trend has not been found, but these are in the minority. Elliott and Marmot (1984) investigated the changes in blood pressure with age and between populations. They found that the changes were consistent with an environmental factor, such as salt intake, affecting the position of the mean of the blood pressure distribution and with a gradient of susceptibility within a population.

Epidemiological evidence concerning dietary potassium intake and blood pressure is not as extensive as that for sodium. Indirect evidence from epidemiological studies supports the protective role of potassium against the 'toxic' effects of excess sodium and vice versa the permissive effect of reduced potassium intake on the hypertensive effect of sodium. Such a protective effect of a high potassium intake against the harmful effects of sodium has been observed in Japanese farmers eating large quantities of apples (Sasaki et al, 1959). Ueshima et al (1981) found a negative correlation between serum potassium and the prevalence of hypertension in six populations.

Since the lowest socio-economic status in Western countries is associated with the lowest potassium intake (lack of expensive fresh fruits and vegetables) this might partially explain the higher prevalence of hypertension in black populations than in whites in some parts of the U.S.A. (Grim et al, 1970) and in South Africa (Manning et al, 1974).

1.5.2 Normal volunteer studies

Miller et al (1987) reported a study which related the blood pressure of normotensive parents to that of their offspring during

sodium restriction. Mother-offspring resemblance in blood pressure change with sodium restriction was significant for systolic, diastolic and mean arterial pressures. Sibling-sibling and twin-twin resemblances were also highly significant for changes in all three blood pressures. Father-offspring resemblances were marginal ($p < 0.1$). The average population pressure was slightly, but significantly lower during the sodium restriction period. In a study looking at the effect of sodium supplementation, Ghione et al (1986) observed no change in blood pressure or cardiac output during short term (3 days) of high salt intake in young normotensive volunteers (15g NaCl was added to normal daily diet), though body weight increased and plasma renin activity decreased significantly. A study by Gudmundsson et al (1984) also showed this to be the case in a longer study. After four weeks of increased salt intake (12g NaCl added to normal daily diet) blood pressures remained unchanged in groups of normotensive volunteers both with and without familial predisposition to hypertension. Thus there seems to be no convincing evidence from acute studies that high salt intake affects the blood pressure of normotensive humans. It seems likely that elevated sodium intake is required over longer periods of time before adverse cardiovascular effects are seen especially as sodium restriction does appear to have a hypotensive effect in such subjects.

1.5.3 Hypertensive patient studies

Evidence supporting Dahl's theory that it is salt intake rather than weight reduction 'per se' that is responsible for the hypotensive effect of weight-reducing diets includes a study by Fagerberg et al (1984). This showed that whilst a moderate

weight-reducing diet decreased indices of sympathetic nervous system activity, reduction of blood pressure to the normotensive range was observed only when there was a concomitant restriction of sodium intake. However, studies by Reisin & Frohlich (1982) have reported that weight loss obtained with a hypocaloric diet without reduced salt intake effected a considerable fall in systolic and diastolic blood pressures in obese hypertensive patients. The mechanisms proposed for this pressure reduction were a decreased sympathetic activity associated with a reduced participation of the renin-angiotensin-aldosterone system, natriuresis, contracted plasma volume and a reversal of the high cardiac output state.

However, not all hypertensive patients are overweight and so studies of salt/sodium restriction without caloric restriction must be considered. Grobbee and Hofman (1986) reviewed 13 randomised trials and found that with a mean reduction in sodium intake of 78mmol per day, there was a fall in blood pressure, which was related to the level of the initial pressure and to the age of the patients. Their figures indicated a significant effect among those who represented the majority of patients in need of therapy, those with definite hypertension who were above the age of 40 years.

The role of dietary potassium in the genesis, therapy and prevention of hypertension has not been investigated as thoroughly as that of dietary sodium. However, studies published to date have shown that blood pressure tends to be related to the urinary Na-K ratio (Langford & Watson, 1975). Os et al (1986) were able to show only a small additional reduction in blood pressure with extra potassium (250mmol K^+ daily in diet) added to an established low sodium regimen (29mmol Na^+ daily), in patients with mild essential hypertension.

However, a study by Siani et al (1987) reported a long term reduction in blood pressure in mildly hypertensive subjects given moderate oral potassium supplements (24 and 48 mmol K^+ daily).

1.5.4 Animal studies

In animals, excess sodium in the diet can increase arterial blood pressure, particularly when the excretion of sodium is impaired. Smith-Barbara et al (1980) showed that a high salt (NaCl) diet induced elevation of blood pressure in Sprague-Dawley rats and that this effect was most marked in rats in which the diet was also high in saturated fat content.

Rats have been inbred to produce strains which become severely hypertensive when fed a high-salt diet, without the need for deliberate reduction in the ability of the kidney to excrete sodium. When the kidneys of these salt-sensitive rats are transplanted into others who are resistant to salt and naturally normotensive, those animals then become hypertensive. Thus, the potential for salt-induced hypertension in these genetically salt-sensitive rats seems to reside, at least in part, in the kidney (Bianchi et al, 1982). Spontaneously hypertensive rats (SHR) of the Okamoto strain have a greater salt appetite than normotensive Sprague-Dawley rats (Di Nicolantonio, 1987). Further, when SHR pups were cross-suckled on Sprague-Dawley rats their salt appetite and blood pressure was significantly lower than in SHR pups suckled on SHR. Thus, it appears that some factor transferred from the SHR mother postnatally is required for full expression of the elevated blood pressure seen in SHR.

The effect of supplementary potassium on the course of

hypertension has been investigated in several experimental models of hypertension. Tobian et al (1986) showed a marked reduction in death rate, brain haemorrhage and infarcts and mesenteric arteriolar hypertrophy in stroke-prone spontaneously hypertensive rats when they were fed a high-potassium diet. These effects were produced without significant reduction in blood pressure. In spontaneously hypertensive rats supplementary potassium lowered blood pressure when they were given diets containing large amounts of sodium (Aoki & Yamori, 1972). In Dahl salt-sensitive rats feeding of supplementary potassium prevented the rise in blood pressure normally induced by a high sodium (4.5%) diet (Meneely et al, 1957).

1.5.5 Mechanisms of action : Theories

a) Mechanism(s) of the pressor response to dietary sodium

One theory by Blaustein (1977) proposed that the correlation between sodium metabolism and hypertension could be accounted for if a circulating agent, perhaps the 'natriuretic hormone', were to affect the electrochemical sodium gradient across the vascular smooth muscle cell plasma membrane (sarcolemma), thus altering the intracellular ionised calcium concentration and hence the resting tension or tone of the vessel. Any change in the sodium gradient would be reflected as a change in intracellular calcium concentration and therefore in vessel wall tension and peripheral resistance.

However, Gavras (1986) postulated that sodium exerts its hypertensive action centrally by decreasing the state of affinity of the α_2 -adrenergic receptors (mainly at the sites of cardiovascular regulation in the brainstem, such as the nucleus tractus solitarius and the locus coeruleus) for locally occurring

agonist neurotransmitters (such as noradrenaline and adrenaline), which would result in disinhibition of sympathoinhibitory neurones and lead to the hyperadrenergic state characteristic of salt-induced hypertension.

b) Mechanism of the hypotensive effect of dietary potassium

Haddy (1987) proposed the following mechanism to account for the hypotensive effect of dietary potassium. As the Na^+, K^+ -ATPase and hence the $\text{Na}^+ - \text{K}^+$ -pump of the vascular smooth muscle cell is sensitive to the extracellular potassium concentration, an increase in extracellular potassium concentration would stimulate the Na^+, K^+ -ATPase and the pump. Such stimulation would cause vasodilation by hyperpolarising the cell. Thus, potassium may be beneficial by causing vasodilation via direct action on the smooth muscle cell. Potassium may also be beneficial by virtue of the diuresis it may cause. It may also cause vasodilation indirectly, by stimulating the Na^+, K^+ -ATPase in the adrenergic nerve terminal and hence increasing the uptake of noradrenaline, thereby leaving less in the neurovascular cleft. Potassium also suppresses plasma renin activity and limits the increase in renin concentration caused by sodium restriction.

1.5.6 Other electrolytes implicated in the control of blood pressure

a) Chloride

The majority of sodium ingested by man is in the chloride form. When discussing the effect of sodium on blood pressure and other functions salt or sodium chloride is usually used as a measure of sodium intake. However, chloride may influence blood pressure independently of sodium. Kurtz et al (1987) showed that oral

administration of sodium chloride (240mmol per day) for 7 days to men with essential hypertension (in whom blood pressure was normal with restriction of dietary sodium chloride to 10mmol per day) induced significant elevation of both systolic and diastolic blood pressure. An equimolar amount of sodium, given as sodium citrate, induced no change in blood pressure however. Both salts induced substantial and comparable sodium retention, weight gain and suppression of plasma renin activity and plasma aldosterone, but supplemental sodium chloride increased plasma volume and urinary excretion of calcium, whereas sodium citrate did not.

b) Calcium

The use of calcium channel blocking agents in the treatment of hypertension suggests an important role for calcium in the biochemical control of vascular smooth muscle tone and thus of blood pressure homeostasis. The relation of the clinical aspects of calcium metabolism, such as calcium intake, renal disposition of calcium and circulating levels of calcium regulating hormones, as well as of calcium itself remains an ambiguous one.

In a study using 3-week-old weanling spontaneously hypertensive rats, calcium supplementation caused growth retardation within 1-2 weeks of treatment compared to controls not receiving the calcium supplement. Prolonged treatment with the high calcium diet completely abolished further increases in blood pressure from 9 weeks of age (the study was continued to 12 weeks), irrespective of concomitant sodium supplementation. Angiotensin-stimulated blood pressure was also attenuated by the high calcium diet regardless of dietary sodium, similar to basal readings (Lau et al, 1986).

McCarron & Morris (1985) showed that treatment with calcium

tablets (1000mg Ca^{2+} per day) for 8 weeks produced a reduction in standing systolic arterial blood pressure in 44% hypertensives and 19% normotensives. However, some population surveys show a positive relationship between blood pressure and serum calcium levels (Kesteloot & Geboers, 1982) and increased intracellular free cytosolic calcium levels have been reported in hypertensive persons (Erne et al, 1984). These studies tend to portray calcium as a pathogenic or exacerbating factor in hypertensive disease. On the other hand, an inverse relationship has been observed between dietary calcium intake and blood pressure (McCarron et al, 1984), which suggests that a relative or absolute calcium deficiency is present in hypertension. Clinically, the findings that some, but not all, hypertensive patients are "salt-sensitive" indicates the heterogeneity of the condition of human hypertension (Resnick, 1985). Therefore, it seems reasonable to expect a similar heterogeneity with respect to calcium metabolism in hypertensive patients. Therefore, the seemingly contradictory findings of various studies of calcium metabolism in hypertension may simply reflect the wide spectrum of blood pressure responses to calcium.

1.5.7 Other possible dietary factors in hypertension

a) Dietary fat and dietary fibre

The role of dietary fat in human hypertension was demonstrated by two intervention studies (Iacono, Dougherty & Puska, 1986) conducted in Beltsville, MD and in North Karelia, Finland. Healthy people served as subjects for the studies and foods consumed by the subjects were typical for the regions. During the intervention periods the percentage of energy from dietary saturated fats was

lowered, while the polyunsaturated fatty acids were increased. Total energy intake and salt intake were kept relatively constant. Systolic and diastolic blood pressures were decreased during the lowered saturated fat/higher unsaturated fatty acids intake period and this effect was reversed during the switchback period. The data give strong support to the hypothesis that high dietary saturated fat content is associated with elevated blood pressure.

In a study using rabbits, blood pressure was always increased by a fat-enriched diet, this effect was diminished and delayed by adding cellulose to the diets, though cellulose itself had no effect on the blood pressure in the absence of fat (Burstyn & Husbands, 1980). This indicates that dietary fibre may also play a role in the control of blood pressure. This possibility was investigated by a study based on a dietary survey and blood pressure measurements (Wright et al, 1979) which classified subjects according to their fibre intake. Subjects with a high fibre intake were found to have lower mean blood pressures than those with a low fibre intake. It is possible that the typically low fat/high fibre diet of the lacto-ovo-vegetarian is partly responsible for the lower blood pressure levels observed in this group of people (Beilin, 1986; Beilin & Margetts, 1987).

b) Dietary alcohol

It has become evident from a series of epidemiological studies that an association exists between regular use of large amounts of alcoholic beverages and hypertension. In most studies, regular intake of smaller amounts of alcohol is not associated with hypertension (Klatsky & Friedman, 1984) but a possible threshold can not be precisely defined at present. The relationship between alcohol intake and blood pressure is not attributable to demographic characteristics

of obesity, reported salt use, smoking or coffee use, nor can it be explained by underreporting of alcohol consumption. If the relationship is causal then the pathogenesis is not yet firmly established (Klatsky & Friedman, 1984).

1.6 CAFFEINE

1.6.1 Introduction

Although the main area of risk in obesity is in the cardiovascular field (see section 1.2.3b)), 'ad libitum' intake of tea and coffee (which may also have adverse cardiovascular effects, see below) is often advised for those following a calorie-restricted diet in preference to more calorie-dense drinks such as milk, soft drinks or alcohol.

Epidemiological evidence for the adverse cardiovascular effects of chronic caffeine consumption are not conclusive for healthy adults (see section 1.6.3), however, the effects in patients with existing cardiovascular disorders such as hypertension might be expected to be more serious (Goldstein & Shapiro, 1987). In addition, it is known that hypertension is more prevalent in the obese (Dustan, 1983; Landsberg, 1986). Hypertension may be asymptomatic when mild or moderate and thus the condition may not be recognised in the obese patient. When an obese person attempts to lose weight (often without medical supervision), by following a calorie-restricted diet, he/she might consume more caffeine than usual, as many of the popular commercial diets (Eyton, 1982; Greatbatch, 1984) state that tea, black coffee and low calorie cola drinks may be consumed freely. If this is the case, then the increased caffeine consumption might have a detrimental effect in these subjects.

A dietary questionnaire was designed to investigate the consumption of caffeine-containing beverages in dieters and non-dieters (see section 2.5) and the results (section 3.5) showed that a proportion of the sample did, indeed, increase their consumption of tea and coffee when dieting.

The effects of two doses of caffeine in both lean and dietary obese rats were studied using both 'in vivo' and 'in vitro' methods to investigate possible differences in cardiovascular effects between the groups (see section 2.8.7 and 3.6).

1.6.2 Dietary intake of caffeine in man

Coffee and tea consumption account for a major part of dietary caffeine intake in man (see Appendix 3 for the caffeine content of popular beverages).

a) Coffee

The first use of coffee, and the discovery of its effects in reducing fatigue and increasing wakefulness, is attributed apocryphally to an Ethiopian holy man. The story goes that he had been told by a herdsman that goats that had eaten the berries of the coffee bush frisked all night long. This led him to prepare a beverage from the berries to enable him to stave off sleep so that he could spend his nights awake and in prayer.

Coffee was introduced into Western Europe at the end of the 16th century, where it spread rapidly in popularity. The demand was so great and the profit from the coffee trade so attractive, that plantations were started in the territories of all the colonial powers where conditions were suitable for cultivation of coffee. The annual production of coffee is now about 4.5 million tonnes. Slightly

less than half the total production is consumed in the U.S.A., where the daily consumption 'per capita' averages about 25g, which is equivalent to over 250mg caffeine. The figure for the U.K. (Ministry of Agriculture, Fisheries and Food, 1986) in 1986 was quoted as 1.74oz per week, which is equivalent to approximately 7g per day (approximately 70mg caffeine per person daily).

Although there is no denying the magnitude of its popularity, opinions have always been divided on the benefits of coffee, as can be seen by the following quotations(both from Bowman & Rand,1980): the first from an advertisement for a London coffee-house in 1652, claimed it "quickens the spirits and makes the heart lightsome...is good against sore eyes...excellent to prevent and cure the dropsy, gout and scurvy... neither laxative nor restringent (ie.constipating)"; the second from Sir Thomas Clifford Allbutt, the Regius Professor of Medicine at Cambridge in the early 1900's, claimed of the coffee-drinker that " the sufferer is tremulous and loses his self-command; he is subject to fits of agitation and depression...He has a haggard appearance... As with other such agents, a renewed dose of the poison gives temporary relief, but at the cost of future misery".

b) Tea

A beverage made by mixing prepared tea leaves with water has been used in China for at least the last 1500 years. Tea also reached Europe in the 16th century. The largest importers of tea are the British, and the annual consumption 'per capita' in the U.K. is about 4.5kg, which is equivalent to over 300mg of caffeine daily. Tea drinking is an integral part of the cultural pattern in many countries, and in Japan it is highly ritualized in the tea-ceremony.

Amongst British people, the rituals are less formal and the characteristics of dependence are more clearly evident. The frank recognition of tea as a drug is shown by the following quotation from the pioneer British pharmacologist W.E.Dixon in the early 1900's (from Bowman & Rand, 1980): "Tea has appeared to us to be especially efficient in producing nightmares with... hallucinations which may be alarming in their intensity... Another peculiar quality of tea is to produce a strange and extreme degree of physical depression. An hour or two after...tea has been taken...a grievous sinking...may seize upon a sufferer, so that to speak is an effort. The speech may become weak and vague...By miseries such as these, the best years of life may be spoilt".

Other minor sources of caffeine in the diet include chocolate, cocoa, cola drinks and compound analgesic preparations which often contain caffeine.

1.6.3 Cardiovascular actions of caffeine (see Fig.2 for structure)

The seemingly contradictory reports of the effects of caffeine on haemodynamic variables clearly show the problems associated with failure to distinguish between habitual consumers and non-consumers of caffeine. Caffeine has been reported to raise (Robertson et al, 1978), lower (Sollman & Pilcher, 1911-1912) and not alter (Starr et al, 1937) arterial blood pressure; increase (Sollman & Pilcher, 1911-1912; Gould et al, 1973), decrease (Horst et al, 1936) and not alter (Starr et al, 1937) heart rate; and raise (Robertson et al, 1978) or not alter (Jung et al, 1981) plasma levels of adrenaline and noradrenaline.

Two relatively recent studies have helped to clarify caffeine's effect on haemodynamic variables. In the first study, caffeine 250mg, was administered to healthy, caffeine-naive subjects using double-blind crossover protocol (Robertson et al, 1978). Systolic blood pressure increased by 10mmHg one hour after ingestion of caffeine. Heart rate decreased during the first hour and then rose above baseline levels for the next two hours. Plasma adrenaline, noradrenaline and plasma renin activity increased after the ingestion of caffeine. A second study examined the effects of chronic caffeine ingestion and used healthy habitual coffee drinkers as subjects (Robertson et al, 1981). The subjects abstained from caffeine-containing beverages for three weeks prior to the start of the trial and they were then given caffeine 250mg or placebo three times daily for 7 days in a randomised double-blind trial. The initial administration produced haemodynamic effects similar to those noted in the first study, however complete tolerance developed to all these effects after 1 to 4 days of caffeine administration. Thus, it now seems quite certain that although the acute ingestion of caffeine alters many haemodynamic variables, the chronic ingestion of caffeine has little or no effect on blood pressure, heart rate, plasma renin activity and plasma catecholamine levels in normal subjects due to the rapid development of tolerance. However, the effects of caffeine on blood pressure may not be abolished with tolerance in older hypertensive subjects, as a study by Goldstein and Shapiro (1987) showed that caffeine (200mg) produced an increase in both systolic and diastolic blood pressure (8/6mmHg) in male hypertensives, who were habitual coffee drinkers (subjects were asked to abstain from caffeine-containing substances for only 12 hours, overnight, prior to

the monitoring sessions).

The effects of chronic caffeine intake, as represented by coffee consumption, upon blood pressure has been reported in a recent Italian study by Periti et al (1987). They were able to show a mean reduction in both systolic and diastolic blood pressure per cup of coffee per day, even after correcting for habitual alcohol intake and cigarette smoking.

The effects of chronic caffeine ingestion on the incidence of coronary heart disease has been studied in epidemiological studies using coffee consumption as a measure of caffeine intake. Again, the reports are contradictory. Dawber et al (1974) presented observations from the Framingham study and concluded that there was no association between the level of coffee consumption and the incidence of coronary heart disease. However, a prospective study of 1130 male medical students by La Croix et al (1986) showed an independent dose-responsive association of coffee consumption with clinically evident coronary heart disease, consistent with a two to three-fold elevation in risk among heavy coffee drinkers.

A study by Dobmeyer et al (1983) on the effects of caffeine on arrhythmogenesis showed that caffeine ingestion tended to promote supraventricular tachycardia in patients with heart disease and in normal controls and ventricular tachycardia in patients only. The conclusion from the study was that patients with heart disease should avoid caffeine.

1.6.4 General pharmacology of caffeine

Caffeine is probably the most widely consumed drug in Western societies. Its pharmacological effects are thus of great interest.

Although caffeine exerts effects on many organs, most published data relate to acute effects. These effects are described below.

The primary effect of caffeine is central nervous system stimulation, acting chiefly on the cerebral cortex. This is expressed in terms of increased vigilance, relief from fatigue, improved mood and improved performance (Curatolo & Robertson, 1983). The effects of caffeine, particularly on mood, seem to be influenced strongly by individual variations in sensitivity or acquired tolerance. Caffeine-attributed insomnia appears to show significant individual variability and it has been proposed that the rate of caffeine metabolism is a determining factor (Levy & Zylber-Katz, 1982). The acute ingestion of caffeine produces mild increases in urine volume and urinary sodium excretion in humans. Tolerance to these effects develops after habitual consumption of the drug (Eddy & Downs, 1928). Acute doses of caffeine produce a number of effects on metabolism, including an increase in metabolic rate (Jung et al, 1981), elevation of plasma levels of free fatty acids and a slight elevation of plasma cortisol levels (Avogaro, 1973). These metabolic effects were found to occur also in habitual coffee drinkers, therefore it appears that tolerance occurs less readily to these effects. The cardiovascular effects of caffeine have been discussed in section 1.6.3.

At the doses commonly encountered in foods and beverages (see Appendix 3), caffeine probably exerts most of its pharmacological effects through antagonism of adenosine receptors (Fredholm, 1980). Adenosine acts as a neurotransmitter in purinergic neurones, which have diverse functions throughout the body (Burnstock, 1972). Receptor antagonism provides a mechanism for the development of tolerance which has been reported to occur with chronic caffeine

treatment. Tolerance to the long-term presence of caffeine is thought to be acquired by increasing the number of adenosine receptor sites and thus the sensitivity to adenosine (Fredholm, 1980). Several other mechanisms of action have been proposed for caffeine including phosphodiesterase inhibition (Beavo et al, 1970) and calcium mobilization (Smits et al, 1986). However, these effects only occur at higher doses of caffeine than would normally be encountered from dietary intake. High doses of caffeine induce a stress-like neuroendocrine response in rats, characterised by increased serum corticosterone and beta-endorphin levels and decreased serum growth hormone and thyrotropin levels (Spindel et al, 1980). However, few or no endocrine effects would be expected in man with the doses that are typically consumed.

The effects of caffeine overdose, which generally occur with intakes greater than approximately 7-8mg/kg daily or 500-600mg daily in adult humans, are generally described in a clinical condition known as "caffeinism". Symptoms that occur include anxiety and tension, headaches, insomnia, diarrhoea, irritability, loss of appetite, dizziness and decrease of hand steadiness (Abbott, 1986). Estimates of a fatal dose of caffeine in man are put at greater than 10g (140mg/kg) (Abbott, 1986). A review of the pharmacology and toxicology of caffeine by Abbott (1986) indicated that there was no positive evidence of adverse carcinogenic, mutagenic or teratogenic effects of caffeine in man.

1.7 THE USE OF RAT MODELS FOR INVESTIGATING CARDIOVASCULAR PARAMETERS

1.7.1 'In vitro' techniques

a) Isolated perfused heart

This method is based upon the isolated perfused rabbit heart preparation first described by Langendorff (1895) and later by Brodie and Cullis (1908) and Gunn (1913). It is a useful preparation for studying cardioactive drugs, as the effects on contractility (force of contraction), rate of beating, perfusion pressure (reflecting the state of dilation or constriction of the coronary vessels if the flow rate of perfusate is kept constant) and arrhythmias can be detected. This was one of the preparations used in some of the studies described here and full experimental details may be found in section 2.7.3.

b) Isolated perfused mesenteric vasculature

This preparation was first described by McGregor (1965). It may be used to study the actions and interactions of sympathetic nerve stimulation and drugs on the mesenteric blood vessels. The combined use of nerve stimulation, agonist drugs and selective antagonists can be used to investigate both presynaptic and postsynaptic activity (Draper et al, 1986). If perfused with Kreb's solution, the preparation may be used to study contractile responses (McGregor, 1965). If perfused with a vasoconstricting agent such as phenylephrine or methoxamine (both approximately 10^{-4} M) to induce tone it may be used to study relaxation responses (Byfield, 1986). This preparation was used in some of the studies described here and experimental details may be found in section 2.7.2.

c) Aortic spiral strip

This preparation is based on that described by Furchgott and Bhadrakom (1953) for the rabbit aortic strip. The preparation requires equilibration under tension in an organ bath for at least an hour to achieve full relaxation before the addition of any drugs (Furchgott & Bhadrakom, 1953). It then possesses negligible intrinsic tone and does not exhibit any spontaneous contraction, making it suitable for studies with vasoconstrictor substances. The cutting of the preparation into a spiral necessarily involves a considerable amount of handling and possibly destruction of the vascular endothelium to some degree. It is not possible to determine how much, if any, of the endothelium has been destroyed without the use of histological staining techniques. It has become apparent that several endogenous vasoconstrictors, including noradrenaline and 5-hydroxytryptamine, release a vasodilator substance from endothelial cells that in turn acts as a physiological antagonist of smooth muscle contractility (Cocks & Angus, 1983). In the absence of endothelium these vasoconstrictors have much greater maximum efficacy. The vasodilator substance released from the endothelial cells may be the same endothelium-derived relaxing factor (EDRF) that is involved in the relaxation response of vascular smooth muscle to acetylcholine (Furchgott & Zawadzki, 1980). Variation in the endothelial integrity of isolated vessels could result in inconsistency in apparent sensitivity to these vasoconstrictor substances when they are applied exogenously. Thus, this preparation was only used for one series of experiments (see section 2.7.1) due to problems with interpretation of the data in the light of these factors.

d) Isolated perfused hindquarters

The perfused rat hindquarters preparation, in addition to its use for studying vasoactive agents (Essmaili-Shad, 1985) has also been used as a skeletal muscle preparation for the study of glucose uptake (Robinson & Harris, 1959; Ruderman et al, 1971). The preparation is thought to possess predominantly postsynaptic beta-adrenoceptors and has been used to study the effects of chronic beta-adrenoceptor blockade (Essmaili-Shad, 1985). This preparation has the practical disadvantage that it rapidly becomes oedematous. Perfusion of the preparation with McEwens solution helps to delay oedema formation, however the preparation is still not viable for long periods.

e) Isolated perfused caudal artery

The isolated caudal artery of the rat is being increasingly used for the study of changes in the vasculature induced by hypertension (Hicks et al, 1984; Fouda et al, 1985) and age (Oster et al, 1988). Proximal segments of the caudal artery are removed, whilst the rat is anaesthetised (usually with pentobarbital), cannulated at both ends and perfused/superfused with Kreb's solution. The degree of vasoconstriction may be determined by changes in perfusion pressure. Responses may be elicited by drugs and/or electrical stimulation. This technique would have been suitable for use in these studies, though the use of the barbiturate anaesthetic (Atkinson et al, 1986) might have caused problems with other preparations taken from the same animal (such as the isolated heart and mesenteric vasculature).

f) Venous smooth muscle preparations

Vascular smooth muscle is heterogeneous in its activity. The effect of a vasoactive agent such as noradrenaline may range from

vasodilation to intense vasoconstriction of different segments of the vascular system (Somlyo et al, 1965).

Human saphenous vein spirals have been used to study responses to sympathomimetic amines such as noradrenaline, adrenaline, phenylephrine, isoprenaline and also 5-hydroxytryptamine (Coupar, 1970). Most of these responses have been shown to be abolished by alpha-adrenoceptor blockers (Coupar, 1970), thus indicating the presence of alpha-adrenoceptors in this preparation. A study using ring segments of rat femoral vein has also demonstrated the presence of alpha-adrenoceptors (postjunctional α_2 -subtype) (Downing et al, 1986).

g) Comparison of methods and factors influencing the choice of preparations used.

Three 'in vitro' preparations were selected for use in the experiments described here; the isolated perfused heart, the isolated perfused mesenteric vasculature and the aortic spiral strip. The isolated heart was selected as a method for assessing cardiac function. The mesenteric vasculature was chosen because obvious physical changes occur in this tissue with the development of obesity namely a large increase in adipose tissue. Also, it is a major vascular bed and any changes in responsiveness of this tissue would be expected to have effects throughout the cardiovascular system. The aortic spiral preparation was used in initial studies as an example of tissue from a major arterial vessel. However work was not continued with this preparation for the reasons stated in section 1.7.1 c).

1.7.2 'In vivo' techniques for the monitoring of cardiovascular function in the rat.

'In vivo' techniques for the monitoring of blood pressure and heart rate may be divided into two categories: invasive, direct methods or non-invasive, indirect methods. Invasive techniques involve the insertion of a cannula into an artery of an anaesthetised rat. Heart rate and blood pressure may then be measured with the animal anaesthetised. Alternatively, the cannula may be secured into place and temporarily sealed and the animal allowed to recover. Direct measurements of heart rate and blood pressure may then be made in the conscious rat via the indwelling cannula. Non-invasive indirect measurement of blood pressure and heart rate may be made using the tail cuff method (see section 2.8.3 for full experimental details).

In order to decide the most appropriate and convenient method for use in these studies it was necessary to consider firstly, that repeated regular measurements were to be made over periods of up to 20 weeks and secondly, that the rats were to be housed in groups, not individually. The first consideration excluded the method using an intra-arterial cannula to measure blood pressure and heart rate in the anaesthetised rat directly. It would not be possible to perform repeated weekly measurements on a single animal over several weeks using this method. Also, the anaesthetic used might affect blood pressure and heart rate. This possibility is important, as larger doses of any lipid-soluble anaesthetic would probably be needed in the dietary obese rats than in lean controls. The second consideration made the method of direct measurement of blood pressure

and heart rate in conscious rats via an indwelling cannula impractical. When rats are housed in groups, they tend to chew through the cannulae of their companions, often with disastrous results. It is desirable to house rats in groups for studies of dietary obesity because, as discussed in section 2.6.2, weight gain is appreciably less if rats are housed in isolation.

Therefore, the indirect tail cuff method was chosen as the most appropriate, because it is relatively simple, reliable, non-invasive and indefinitely repeatable.

1.8 AIMS OF THE EXPERIMENTS

1.8.1 Comparison of various cardiovascular parameters in lean and dietary obese rats throughout the development of obesity.

It is known that obesity is associated with increased incidence of cardiovascular disorders, hypertension in particular (see section 1.2.3 b)). However, the underlying cause or causes of this association have not been clearly identified. Indeed, it is not certain that the relationship between obesity and hypertension is causal rather than coincidental. The aims of these experiments were firstly, to study changes in heart rate and blood pressure in the conscious rat throughout the development of obesity, comparing these values with those of lean age-matched controls. Secondly, 'in vitro' preparations were used to study changes in responses to noradrenaline, acetylcholine and 5-hydroxytryptamine in tissues (isolated heart, isolated perfused mesentery and aortic strip) from obese animals as their obesity progressed and to compare these responses with those of tissues from age-matched lean controls.

1.8.2 Comparison of the effects of weight reduction, induced by dietary restriction or by use of anorectic drugs, on cardiovascular function in the rat.

Weight reduction has been shown to cause a reduction in arterial blood pressure in both hypertensive and normotensive subjects (see section 1.2.3 d)). The use of anorectic drugs might be of help both in the short term, to enable adequate amounts of weight to be lost and in the long term, to ensure that the weight is not regained (see sections 1.2.4 e) and 1.4). However, it is important that any cardiovascular side-effects of the anorectic drugs used do not negate any beneficial effects of weight loss. Any intrinsic hypotensive action of the drug would, of course, be advantageous.

The aim of this part of the work was to compare the cardiovascular function of previously dietary obese rats, who had been treated with amphetamine, fenfluramine or mazindol to induce weight loss, with that of age-matched lean and dietary obese rats and also with rats whose weight had been reduced as a result of withdrawal of the palatable diet for 4 weeks, following an initial 4 week period of receiving the palatable diet to induce dietary obesity.

1.8.3 Analysis of the consumption of caffeine-containing beverages in a sample of staff and students at Bath University and an investigation of some cardiovascular effects of two doses of caffeine in rats with and without the palatable diet.

A dietary survey was designed (see Fig3) and subjects were asked to indicate their normal daily consumption of tea, coffee and various

other items and, if they had ever dieted, to indicate any changes in consumption of these items when dieting. One of the conclusions drawn from this study was that a significant proportion of the subjects increased their tea and coffee consumption (and therefore increased their caffeine intake) when dieting. See section 3.5 for full details of the results of the study.

Two doses of caffeine (25mg/kg and 50mg/kg) were studied in both lean (chow-fed) and dietary obese (palatable diet plus chow) rats. Cardiovascular function in these animals was assessed both 'in vivo', throughout the 4 weeks of the experiment, and 'in vitro' at the end of the study (see sections 2.8.7 and 3.6).

1.8.4 The measurement of plasma sodium and potassium concentrations in lean and dietary obese rats.

Analysis of the food consumed by the rats studied revealed that the sodium content of the food consumed by rats offered the palatable diet (dietary obese) was several times greater than that consumed by the rats offered chow only (lean). Higher dietary sodium intake has been associated with elevated blood pressure (see section 1.5). The dietary obese rats were also found to have a lower intake of potassium than lean rats. As potassium has been reported to have a hypotensive effect and to offer protection against the potentially hypertensive effects of sodium (see section 1.5), this reduced potassium intake might exacerbate any effects of the high sodium content of the palatable diet. The dietary obese rats were found to have significantly higher systolic blood pressures than lean controls (see section 3.1.3). A significant correlation between serum sodium

and systolic blood pressure was found in morbidly obese patients (Nanji & Freeman, 1985). The aim of the study described here was to investigate possible differences in plasma sodium and potassium concentrations between lean and dietary obese rats at different stages in the development of obesity.

1.8.5 Monitoring of systolic blood pressure throughout the oestrous cycle in the rat.

The aim of this study was to see if there was any significant variation in the systolic blood pressure of female Wistar rats throughout the oestrous cycle, which might affect the accuracy of blood pressure monitoring in the studies described here.

Conflicting results have been reported concerning the variation of blood pressure throughout the human menstrual cycle (Phillips, 1968; Greenberg, 1985) and these studies are discussed more fully in section 2.8.4). However, the effect of the oestrous cycle on the rat does not appear to have been considered in the many published studies in which blood pressure has been monitored chronically. This is probably due to the fact that the majority of studies used male rats. Sclafani and Gorman (1977) reported that female rats displayed greater weight gain than males when offered a palatable diet and therefore they are most suitable for studies of dietary obesity. The female Wistar rats of the University of Bath strain are particularly suitable for this type of work because their normal growth curve begins to plateau at 225-250g and thus by starting experiments with the rats at 200g the rats offered the palatable diet rapidly become significantly heavier than the lean controls.

Chapter 2 : METHODS

CHAPTER 2 : METHODS

2.1 GENERAL INTRODUCTION

The majority of the methods described here involved the use of the dietary obese rat model of obesity, monitoring the changes occurring in these animals during the development of obesity and also during the subsequent weight reduction regimens.

As a result of the findings of a survey of the intake of caffeine-containing beverages of dieters and non-dieters, a further set of experiments was devised to investigate the effects of two doses of caffeine in rats, both with and without the palatable diet.

2.2 MATERIALS

2.2.1 Physiological salt solutions used for 'in vitro' experiments.

2.2.1 a) 'Normal' Kreb's solution.

This is basically the solution described by Krebs and Henseleit (1932), but with 9.52mM glucose instead of 10mM, and with 0.03mM EDTA (ethylenediaminetetra-acetic acid), (disodium salt, Sigma) added to chelate any heavy metal ions present.

<u>Quantities (g) per 5 litres</u>	<u>Concentration (millimoles/litre)</u>
NaCl - 34.60	Na ⁺ 143.30
KCl - 1.75	K ⁺ 5.60
NaHCO ₃ - 10.50	HCO ₃ ⁻ 24.90
Glucose - 10.00	Glucose 9.52
MgSO ₄ .7H ₂ O - 1.45	Mg ²⁺ 1.20
CaCl ₂ .6H ₂ O - 2.80	Ca ²⁺ 2.60
KH ₂ PO ₄ - 0.80	H ₂ PO ₄ ⁻ 2.20
	Cl ⁻ 128.30
	SO ₄ ²⁻ 1.20

Aerating gas: O₂ + 5% CO₂

This solution was used for studies involving the isolated perfused mesenteric vasculature and the aortic strip.

2.2.1 b) Modified 'heart' Kreb's solution

The major difference between this solution and the 'normal' Kreb's solution is the lower concentration of Ca⁺⁺, though there are some other minor differences (see below). The formula given below is based on that described by Bentham (1986).

<u>Quantities (g) for 5 litres</u>	<u>Concentration (millimoles/litre)</u>
NaCl - 34.48	Na ⁺ 143.00
KCl - 1.75	K ⁺ 5.90
NaHCO ₃ - 10.50	HCO ₃ ⁻ 24.90
Glucose - 10.45	Glucose 9.95
MgSO ₄ .7H ₂ O - 1.48	Mg ²⁺ 1.22
CaCl ₂ .6H ₂ O - 1.31	Ca ²⁺ 1.22
KH ₂ PO ₄ - 0.94	H ₂ PO ₄ ⁻ 2.59
	Cl ⁻ 104.80
	SO ₄ ²⁻ 1.22

Aerating gas: O₂ + 5% CO₂

2.2.1 c) 'High potassium' (65mM K⁺) Kreb's solution

This solution was used to produce increased tone in the isolated perfused mesenteric vasculature (measured as an increased baseline perfusion pressure), as an example of a non-drug-induced vasoconstriction.

<u>Quantities (g) for 5 litres</u>	<u>Concentration (millimoles/litre)</u>
NaCl - 15.37	Na ⁺ 83.90
KCl - 24.24	K ⁺ 65.00
NaHCO ₃ - 10.50	HCO ₃ ⁻ 24.90
Glucose - 10.45	Glucose 9.95
MgSO ₄ .7H ₂ O - 1.48	Mg ²⁺ 1.22
CaCl ₂ .6H ₂ O - 1.31	Ca ²⁺ 1.22
KH ₂ PO ₄ - 0.94	H ₂ PO ₄ ⁻ 2.59
	Cl ⁻ 104.80
	SO ₄ ²⁻ 1.22

Aerating gas: O₂ + 5% CO₂

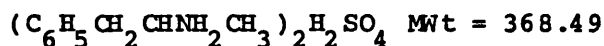
2.2.1 d) 'High potassium/high calcium' Kreb's solution.

This solution was used to perfuse the isolated mesenteric vasculature immediately after the 'high potassium' Kreb's solution, to produce a further vasoconstriction (non-drug-induced). The vasoconstriction was slow to develop and reached a peak after about 20-25 minutes. The solution is the same as that described in section 2.2.1.c) except that it contains additional Ca^{2+} (for 5 litres a total of 3.28g $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$) to give a final concentration of 3.00mM Ca^{2+} . This concentration was chosen because it is known to produce constriction of other pharmacological preparations, such as the taeni coli muscle.

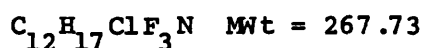
2.2.2 Drugs used

a) Drugs used for 'in vivo' studies

i) Amphetamine sulphate, Sigma



ii) Fenfluramine hydrochloride, Servier



iii) Mazindol, Sandoz



iv) Caffeine, Sigma



(see Figs.1 and 2 for structural formulae of the above drugs)

b) Drugs used for 'in vitro' studies

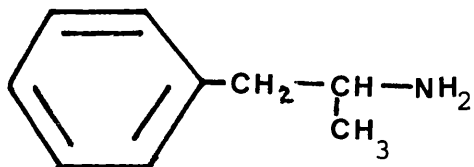
i) Noradrenaline bitartrate, Sigma



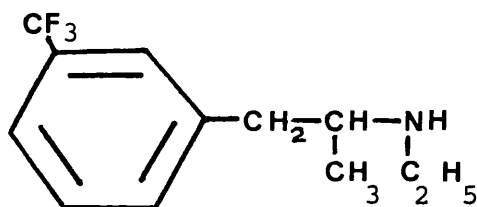
ii) Acetylcholine chloride, Sigma



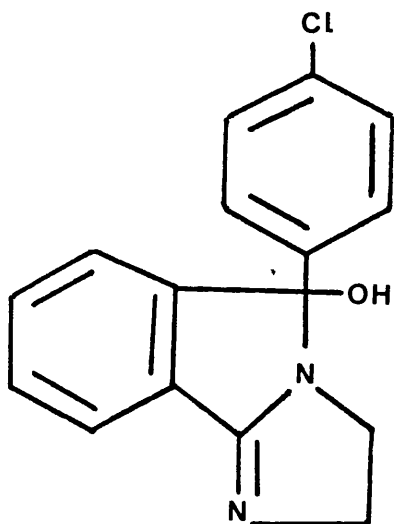
Fig. 1 Structural formulae of anorectic drugs used.



Amphetamine : α -methyl- β -phenylethylamine

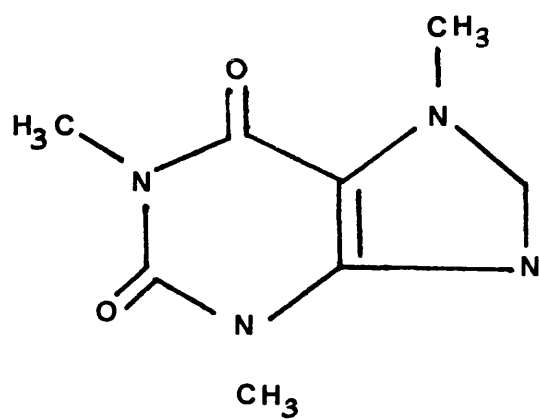


Fenfluramine : 2-ethylamino-1-(3-trifluoromethylphenyl)
propane



Mazindol : 5-hydroxy-5-(4-chlorophenyl)-2,3-dihydro
5H-imidazo-(2,1-a)-isoindole

Fig. 2 Structural formula of caffeine

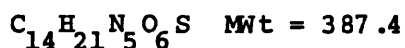


Caffeine : 3,7-Dihydro-1,3,7-trimethyl-1-H-purine,
2,6-dione

iii) Phenylephrine hydrochloride, Sigma



iv) Serotonin (5-HT) creatinine sulphate complex (monohydrate), Sigma



2.3 MEASUREMENT OF PLASMA CONCENTRATIONS OF SODIUM AND POTASSIUM BY FLAME PHOTOMETRY.

The rats were anaesthetised using ether and samples of blood (approximately 2ml) were withdrawn by cardiac puncture. The blood was immediately placed in lithium heparin tubes (to prevent coagulation), centrifuged at 2000 r.p.m. for 10 minutes at a temperature of 4°C and the plasma layer pipetted off. The samples were then refrigerated at 4°C if not analysed immediately.

A Gallenkamp Flame Analyser was used to determine the plasma concentrations of sodium (Na^+) and potassium (K^+). For the determination of potassium concentration, 0.2ml plasma was diluted with 9.8ml deionised water (1:50). For the determination of sodium concentration 0.1ml plasma was diluted with 9.9ml deionised water (1:100). A standard solution containing 141mM Na^+ , 107mM Cl^- , and 5.1mM K^+ , was used to construct calibration curves from which the concentrations of sodium and potassium in the experimental samples could be calculated.

2.4 STATISTICAL METHODS

Differences between mean values of cardiovascular parameters such as blood pressure and heart rate for groups of lean and dietary obese rats were analysed using Student's t-test for grouped (unpaired) data. However, when data from more than one experimental

group was compared with data from the same control group a modified t-test was used, Dunnett's test. This test reverts to a Student's t-test when data for only one experimental group is used. The non-parametric Mann-Whitney U-test was used for analysis of the occurrence of arrhythmias in the isolated heart preparation following doses of noradrenaline. The nature of these particular results made them unsuitable for analysis by parametric tests. The correlation coefficient (r) was used, where appropriate, to assess the degree of correlation between variables. Statistical significance was accepted at $p < 0.05$.

2.5 SURVEY OF THE INTAKE OF CAFFEINE-CONTAINING BEVERAGES BY DIETING AND NON-DIETING ADULTS.

As discussed in section 1.6, many popular low calorie diets advocate 'ad libitum' intake of tea, coffee and 'diet cola' drinks, which might be expected to lead to an increase in caffeine intake when dieting. A survey was designed with the primary aim of determining whether or not people alter their tea and coffee (and thus, caffeine) intake when dieting.

The subjects were staff and students of the University of Bath. 62 completed questionnaires were returned, from 32 male subjects and 30 female subjects. The age range of subjects was 19-63 years (mean = 28.7 years). The questionnaire was constructed so as to obscure the main aim of the survey, thus questions about smoking and snack food intake, as well as drinks, were included (see specimen questionnaire Fig.3). If the subject had never dieted then the table at the bottom of the questionnaire was left blank. If they had dieted, then they

Fig.3 Specimen "Dietary Survey"

DIETARY SURVEY

- 1) Height 2) Weight 3) Age 4) Male/Female
 5) Smoker/Non-smoker (If smoker, please indicate number per day)
 6) Please indicate your normal daily (average) intake of the following:

	Amount
coffee
tea
cola drinks
other soft drinks
milk
water
alcoholic drinks (specify)
other drinks
crisps
salted peanuts
chocolate

- 7) Have you ever dieted in the past? YES/NO

If "YES", please complete the table below.

- 8) When dieting, please indicate any alterations in your normal intake of the following (mark change with an "X").

	DECREASE	NO CHANGE	INCREASE	DAILY CONSUMPTION WHEN DIETING
coffee				
tea				
cola drinks				
other soft drinks				
milk				
water				
alcohol (specify)				
other drinks (specify)				
crisps				
salted peanuts				
chocolate				
cigarettes (if applicable)				

A copy of the above questionnaire was given to each person upon their entry to the refectory of the School of Pharmacy, University of Bath between 12.00 and 13.00 on one day. 65 forms were given out, 62 of which were returned completed. The ratio of male:female respondents of approximately 50:50, is representative of the overall male:female ratio of staff and students within the School.

were asked to indicate any changes in their intakes of the items listed when dieting. All questionnaires were completed anonymously. Subjects were asked to state their age, height and weight and also whether or not they were smokers. Smokers were asked to state the number of cigarettes they smoked daily. Height and weight values were converted to metric measurements where necessary and the Body Mass Index (BMI) of $\text{weight (kg)/height}^2(\text{m}^2)$ was calculated as an indicator of the level of obesity present (see section 1.2.1). The mean values of various different parameters for 'dieters'(that is, those subjects who had dieted at some time) and 'non-dieters'(subjects who had never dieted) were compared using the Student's t-test. Tea and coffee intake of subjects, when dieting and when not dieting were compared. The correlation coefficient (r) was used to investigate associations between various parameters including BMI and age (years); BMI and the number of cigarettes smoked daily; and BMI and the number of cups of coffee consumed daily.

2.6 EXPERIMENTAL DESIGN OF ANIMAL STUDIES

2.6.1 Animals used

Animals used throughout the studies were female Wistar rats, University of Bath strain. Animals were taken for studies 6 weeks after weaning (approximately 9 weeks of age), at weights of between 180 and 220g (unless otherwise stated) and randomly assigned to experimental groups.

2.6.2 Housing conditions

Amario et al (1984) found that, under conditions of crowded housing, with 10 rats per cage, rats showed reduced rates of body

weight gain. Agadah (1985) showed that rats housed in groups of 6 showed a greater rate of weight gain than rats housed individually. Thus, a group size of approximately 6 rats appears to produce optimum weight gain. The rats used in these studies were housed in groups of 6, in cages (56cm x 38cm x 18 cm) with solid floors. All groups were kept under lighting conditions of a 12:12 hours light:dark cycle and at a constant temperature of 22°C (72°F). All rats had 24-hour 'ad libitum' access to water and standard laboratory chow (CRM brand, for nutrient content see Appendix 1). Animals receiving the palatable diet also had 24-hour 'ad libitum' access to these additional food items, which were provided fresh each day (for details of palatable diet see section 2.8.1).

2.7 'IN VITRO' METHODS USED TO ASSESS CARDIOVASCULAR FUNCTION IN THE RAT

2.7.1 Aortic spiral strip

The preparation procedure was based on that described by Furchgott and Bhadrakom (1953) for the rabbit aortic strip. The rat was stunned by a blow to the head and then decapitated by guillotining. The aorta was cut through as close to the heart as possible and dissected free to the level of the diaphragm. It was transferred to a dish containing Kreb's solution (see section 2.2.1a)) and cut spirally to produce a continuous strip about 2mm wide and 20mm long. A thread was then attached at each end and the preparation mounted in Kreb's solution at a temperature of 37°C, aerated with a mixture of oxygen (95%) and carbon dioxide (5%). One end of the strip was attached to a fixed pin in the organ bath and

the other to the lever of an isotonic transducer, connected to a Washington 400 recorder. The weighting on the lever was approximately 1g. The preparation was allowed to equilibrate for one hour before commencing the experiment to ensure that the tissue had relaxed fully. Doses of noradrenaline (dissolved in Kreb's solution, with 0.05% w/v ascorbic acid added as antioxidant) were added to the organ bath and a log dose vs. response curve constructed for each preparation. As the preparation only responded relatively slowly and required a long time to return to resting conditions after each dose, it was necessary to use a long dose cycle. This consisted of 3 minutes contact time, followed by washing out of the drug and 15 minutes recovery time (after the higher doses a recovery period of up to 30 minutes was often required for full relaxation of the strip to resting length). Graphs were constructed and the EC_{50} of noradrenaline (concentration required to produce 50% maximal response) calculated for each preparation. The mean values (\pm s.e.m.) were calculated for tissues from lean (5 rats, offered laboratory chow only) and dietary obese (5 rats, offered the palatable diet in addition to laboratory chow). Student's t-test for grouped data was used to compare the means of values from the two groups. For this preparation tissues from lean and obese animals were studied after 8 weeks of laboratory chow with or without the palatable diet, respectively. Noradrenaline was the only drug used on the aortic strip.

2.7.2 Isolated perfused mesenteric vasculature

The method used was based on that described by McGregor (1965), which involved the perfusion of the isolated mesenteric vascular bed

with Kreb's solution. It may be used to study the contractile response to drugs such as noradrenaline or, if pre-contracted with, for example 10^{-4} M methoxamine, to study relaxant drugs such as acetylcholine (Byfield et al, 1986).

The rats to be used were heparinised (100U/100g body weight), whilst under ether anaesthesia. In early preliminary studies non-heparinised animals were used. However some large blood clots tended to remain in the blood vessels, which might have interfered with responses. Therefore, heparinised rats were used for all the 'in vitro' tissue studies reported here. The rats were killed by cervical dislocation whilst still under anaesthesia. The carcasses were placed in a supine position and the abdomen opened from the diaphragm to the level of the bladder. The intestines were moved to one side and kept moist with cotton wool soaked in Kreb's solution(see section 2.2.1a), whilst the superior mesenteric artery was cannulated (see Fig.4). The large arterial branch to the caecum (colic artery) was tied off before removing the mesenteric vasculature, complete with the surrounding connective tissue, from the small and large intestines. The preparation was mounted in an organ bath (internal measurements 60mm x 38mm x 22mm), maintained at 37°C and perfused with Kreb's solution at 37°C , (see section 2.2.1a) at a rate of 5ml/minute, using a peristaltic pump (see Fig.5 for diagram of apparatus). For investigation of the responses to vasoconstrictor drugs, such as noradrenaline, the tissue was then allowed to equilibrate for 30 minutes, to ensure full relaxation had been achieved, before commencing the dosing cycle. For relaxant drugs such as acetylcholine, after the initial equilibration period, the tissue was then perfused with Kreb's solution containing 10^{-4} M or 10^{-3} M phenylephrine for a

further 30 minutes, until a steady vasoconstriction was achieved (see section 3.1.7).

Drugs, dissolved in Kreb's solution, were then administered to the perfusate as bolus injections via an injection port. The volume of each injection was kept constant at 10 μ l to eliminate the effect of pressure changes induced by the added volume. Responses were recorded, as mmHg, by a Narco Bio-systems Physiograph MK-IV, via a blood pressure transducer. The recorder and transducer were calibrated using a mercury manometer before each series of experiments. Log dose vs. response curves were constructed and the mean values for baseline pressure and maximum response (as increase/decrease in pressure) and ED₅₀ (dose required to produce 50% maximum response) determined. Values for lean, obese, obese-reduced (both drug-treated or by withdrawal of the palatable diet, see section 2.8.6), and caffeine-treated rats (see section 2.8.7) were determined. When the effects of different concentrations of potassium and calcium in the Kreb's solution were being studied, the preparation was perfused with each solution for 30 minutes (see sections 2.2.1a), c), and d)) and the peak pressure, the plateau pressure and the time to reach plateau pressure were recorded for each solution in turn. For comparison of more than one experimental group with one control group the Dunnett's test was used for statistical analysis and where only tissues from lean and dietary obese animals were compared the Student's t-test was used (see section 2.4).

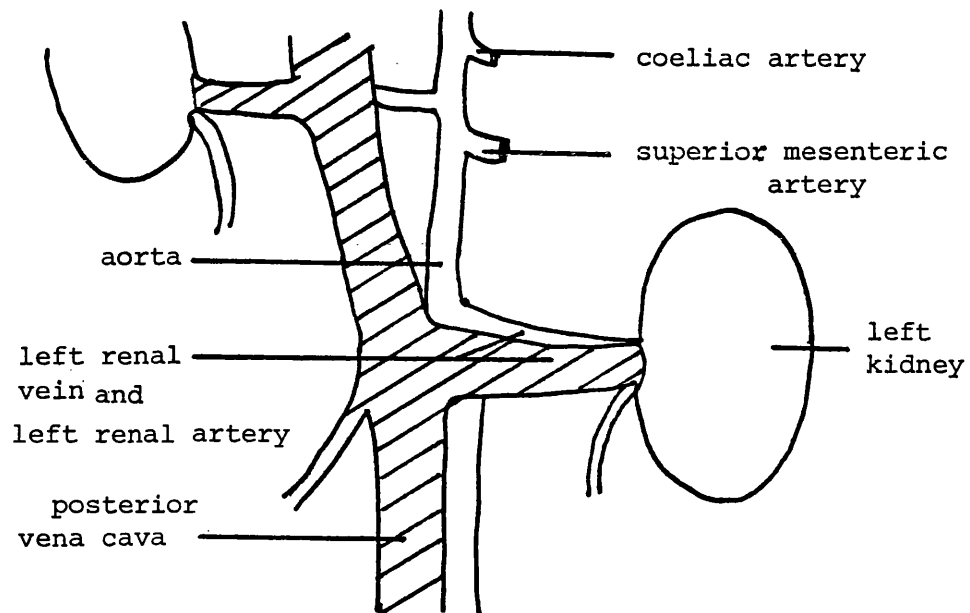
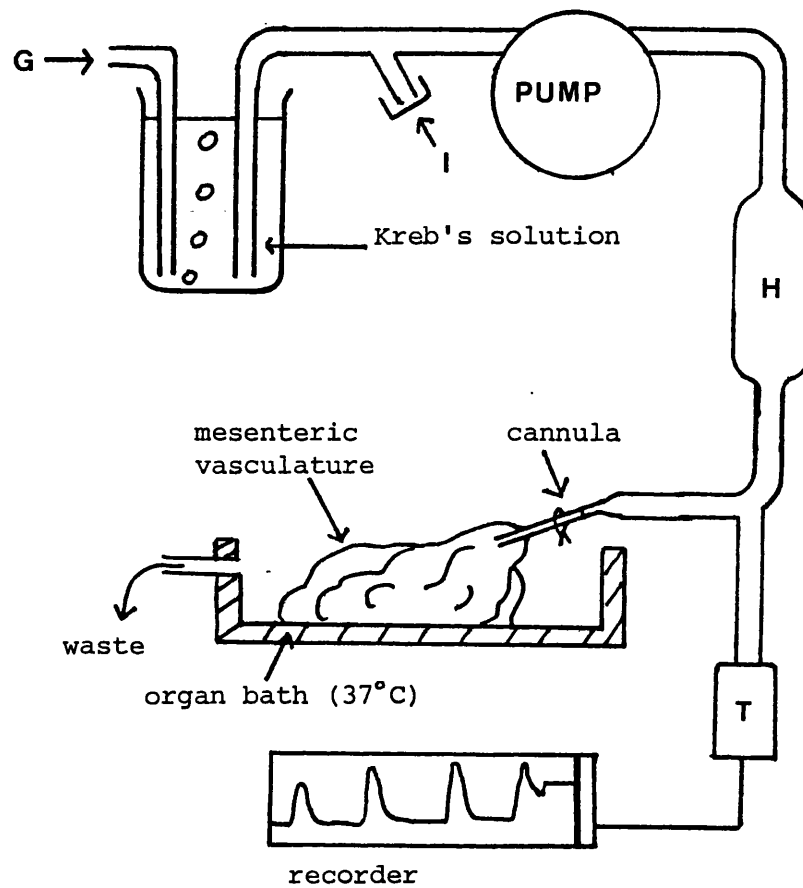


Fig. 4 Diagram showing anatomical location of the superior mesenteric artery.
(adapted from Rowett, 1979)

Fig. 5 Diagram of apparatus for isolated perfused mesenteric vasculature preparation.



Key: G = gas inlet (95% O₂, 5% CO₂)
 I = injection port
 H = heating coil (37°C)
 T = pressure transducer

2.7.3 Isolated perfused heart

This method is based on the isolated perfused rabbit heart preparation, first described by Langendorff (1895) and later by Brodie and Cullis (1908) and Gunn (1913). It is a useful preparation for studying cardioactive drugs, as the effects on contractility (force of contraction), rate of beating and perfusion pressure (reflecting the state of dilation/constriction of the coronary vessels) can be monitored. The occurrence of any arrhythmias can also be detected.

The rat was heparinised and killed, as described in section 2.7.2. The heart was removed immediately, with at least 4-5mm length of aorta still attached and placed in a dish of ice-cold Kreb's solution. The heart was then gently pressed several times to remove as much blood as possible. The aorta was located and dissected free and cannulated with a stainless steel cannula which was connected to the perfusion system (see Fig.6 for diagram of apparatus). The cannula was secured in place with thread. The heart was then perfused with modified Kreb's solution (see section 2.2.1b) at 37°C, applied at a constant rate of 10ml/minute by retrograde aortic perfusion (the pressure of the fluid closes the aortic valve, so the fluid passes only through the coronary vessels and escapes from the inferior vena cava). The heart was enclosed by an open-ended heating jacket maintained at 37°C. A thread was attached to the base of the left ventricle by a small nickel pin-hook and the force of contraction, against a 2g basal tension, was recorded via an isometric transducer, by a Washington 400 recorder. A pressure transducer was connected proximal to the aortic cannula to monitor perfusion pressure, which was again recorded by a Washington 400 recorder. Heart rate was

calculated by monitoring the chart speed for the trace monitoring contractility of the heart. Any ventricular arrhythmias (ectopic beats, missed beats, ventricular fibrillation) were also detected on this trace. Drugs, dissolved in the modified Kreb's solution, were introduced via an injection port proximal to the aortic cannula in a 5 μ l dose volume and the following responses were recorded: contractility (g), heart rate (beats/minute), perfusion pressure (mmHg), ventricular arrhythmias. A 10 minute interval was allowed between each dose (for both noradrenaline and acetylcholine).

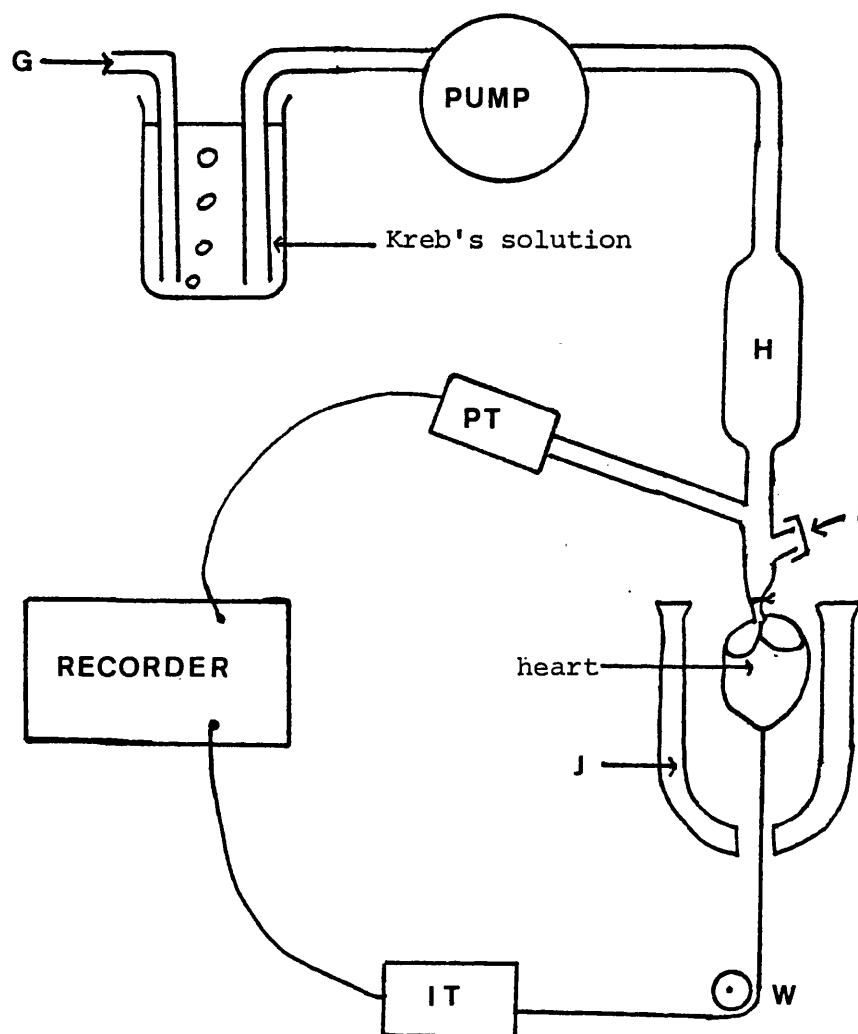


Fig.6 Diagram of apparatus for isolated perfused heart preparation.

Key : H = heating coil (37°C)
 I = injection port
 J = open-ended heating jacket (37°C)
 W = wheel (free-spinning)
 IT = isometric transducer
 PT = pressure transducer
 G = gas inlet (95% O₂, 5% CO₂)

2.7.4 Summary of 'in vitro' studies used

Table 2.7(1) summarises the 'in vitro' preparations and drugs used to assess cardiovascular function in lean and dietary obese rats after different periods of consumption of laboratory chow with or without the palatable diet.

Table 2.7(1)

<u>Preparation</u>	<u>Aortic spiral</u>	<u>Isolated perfused</u>	<u>Isolated perfused</u>
	<u>strip</u>	<u>mesenteric</u>	<u>heart</u>
<u>Weeks</u>		<u>vasculature</u>	
4	X	NAdr 5-HT PE/ACh K^+/Ca^{2+}	NAdr ACh
8	NAdr	NAdr K^+/Ca^{2+}	NAdr ACh
10	X	NAd PE/ACh	X
15	X	5-HT	X
20	X	NAdr	X

Abbreviations used:

NAdr = noradrenaline

5-HT = 5-hydroxytryptamine

PE = phenylephrine (added to Kreb's solution to produce vasoconstriction, prior to dosing with acetylcholine)

ACh = acetylcholine

K^+/Ca^{2+} = Kreb's solution containing 65mM K^+
and 3mM Ca^{2+} (see section 2.2.1)

X = not studied

2.8 'IN VIVO' METHODS USING THE DIETARY OBESE RAT

2.8.1 Production of dietary obesity in the rat

A modified form of the palatable diet used by Sclafani and Springer (1976) was used in the following studies. This consisted of 80g Rice Krispies, 40g digestive biscuits, 40g luncheon meat and 40g chocolate daily, for each group of 6 rats. Laboratory chow and water were also provided 'ad libitum'.

The rats were randomly assigned to groups and housed 6 to a cage as described earlier (see section 2.6.2). The pellets of laboratory chow were placed in the food hopper in each cage. Water was also freely available from 500ml bottles which were refilled each day. Fresh quantities of the palatable food items were placed in the cage daily. The amounts of each food were chosen such that there was always some excess left after 24 hours (see Appendix 1 for the nutrient content of the palatable foods and chow).

2.8.2 Monitoring of food and water intake

The monitoring of water intake was carried out daily by placing 500ml of tap water into a water bottle (using a measuring cylinder) and measuring the volume remaining after each 24 hour period. The volume consumed per rat per day was then calculated. This value was needed when a drug was to be given in the drinking water in order to determine the concentration of the drug required. It was also used to monitor any effects of diet and/or drugs on fluid intake.

The monitoring of intake of laboratory chow was carried out by placing a known weight of chow into the hopper and then weighing the quantity remaining after the 24 hour feeding period. The monitoring of the intake of the palatable food items was difficult if the rats

were housed with the normal sawdust in the base of the cages as food items could be overlooked when collecting the remains at the end of a 24 hour period. For this reason soft blue tissue paper was used to cover the base of the cages, when monitoring the intake of the palatable diet. Weighed quantities of each food were placed in the cage. After the 24 hour feeding period, the remaining food was removed and weighed. Measurements of food intake were then made by calculating the difference between the weights of each item at the beginning and the end of each 24 hour feeding period. Corrections were made for weight loss due to loss of water before absolute food intake values were calculated. It was found that luncheon meat lost 20% of its weight through dehydration during any 24 hour period under the conditions of temperature and humidity in the animal housing rooms, but that there was no effect on the other foods used in these studies.

2.8.3 Cardiovascular monitoring

a) Tail cuff method for measurement of systolic blood pressure.

A study of the limitations of the tail cuff method by Ferrari et al (1986) concluded that systolic blood pressure measurements obtained directly (via an indwelling cannula) and indirectly (tail cuff) were closely similar within groups and that the tail cuff method was therefore technically accurate for studying groups of rats.

A programmed electrosphygmomanometer (Narco Bio-Systems, Texas) was used with an inflatable tail cuff (7/16 inch internal diameter) and a pneumatic sensor and was attached to a recorder (JJ CR650S). The rats were pre-warmed, at $36 \pm 1^{\circ}\text{C}$ for 10 minutes, so that full

dilation of the caudal arteries was maintained throughout the procedure, thus facilitating detection of the pulse (see specimen trace, Fig.9). The rats were transferred from the warming box to a thermostatically controlled heated restrainer ($36 \pm 1^{\circ}\text{C}$). The tail cuff was placed as close to the base of the tail as possible, with the pneumatic sensor held lightly in place immediately distal to the tail cuff. The cuff was then programmed to inflate and deflate at a rate of 25mmHg/second up to a predetermined maximum value of 150-200mmHg (at least 20mmHg higher than the systolic blood pressure of the rat at the last monitoring session). Recording was restricted to a 10 minute period, during which at least 3 readings were obtained. Both the minimum and mean values for each rat, per session, were used for the calculation of mean blood pressure values for each group. The minimum values were used for most calculations and statistical analyses, as it was found that the mean value (for each rat, per session) could be altered considerably by one 'odd' reading, caused perhaps by temporary stress (such as an unexpected loud noise) occurring at some point in the 10 minute monitoring period. In almost all cases the rat was quiet and calm for part of the monitoring period and a reading obtained at this time was usually the lowest value for each monitoring period. It was assumed that this minimum value corresponded, as nearly as possible, to the blood pressure value which would be obtained if the animal was unrestrained.

The effects of heating and restraint, which are inherent to this method, need consideration. The temperature of the pre-warming box, and the length of time each rat spent in the box, were strictly controlled ($36 \pm 1^{\circ}\text{C}$ for 10 minutes). These values were chosen as the result of early preliminary studies as being the conditions which

gave clear, reproducible readings at the lowest temperature, using a fairly short warming time. The temperature of the restrainer and the length of time each rat spent in restraint whilst readings were being taken, were also kept constant ($36 \pm 1^{\circ}\text{C}$ for 10 minutes). This length of time was chosen because the minimum blood pressure reading (which it was assumed corresponded to the 'least stressed' value) for each rat, in any one recording period was found to occur within the first 10 minutes after being placed in the restrainer. Some of the animals required almost 10 minutes to acclimatise to the conditions before their blood pressure reached its lowest value. Other animals, however, gave a minimum reading early in the recording period and became restless, with an increase in blood pressure, with prolonged restraint.

b) Monitoring of heart rate

Again using the tail cuff method (see previous section), heart rate was monitored at the start of each blood pressure monitoring period and again at the end. A faster chart speed (10mm/sec) was used to facilitate these readings. The mean of the two values obtained for each rat, during any one recording period, was used for statistical analysis.

c) Pre-test conditioning

The pre-warming procedure, required for the tail cuff method of monitoring heart rate and blood pressure, may cause some stress for the rats. The restraint necessary, during the actual recording period may also be a cause of stress. The animals were all subjected to at least three monitoring sessions during the week prior to the start of each study, in order to accustom them to being handled and to the warming and monitoring procedures. A progressive reduction in both

blood pressure and heart rate values was observed during this pre-treatment conditioning week, after which the values stabilised.

2.8.4 The effect of the stage of the oestrous cycle on blood pressure in lean and dietary obese rats.

Research concerning blood pressure variation during the menstrual cycle has yielded conflicting and non-conclusive results. In a review by Phillips (1968), some reported a cyclical variation in blood pressure, with a maximum occurring during the week prior to menstruation (Jacobi, 1876; King, 1914), however, in some studies the variation was found to be no greater than that occurring by chance (Moore & Cooper, 1923), while others concluded that there was little or no significant change in blood pressure throughout the cycle (Griffith, 1929). A more recent publication by Greenberg (1985) demonstrated a significant elevation in blood pressure during days 17-26 of the cycle than during the other days of the cycle in one study. However, the results of a second study (reported in the same paper) showed no cyclical change in blood pressure levels.

Many of the animal studies reported here involved the regular, repeated measurement of the blood pressure of groups of female rats over several weeks. The values obtained were compared both within the groups (changes with time) and between groups (changes with drug treatment or dietary manipulation). Thus, it was apparent that the rats would be at different stages of the oestrous cycle when the readings were made. Therefore, it was considered important to determine whether there were any oestrous cycle-linked changes occurring in the blood pressure of the rats before any other studies were commenced. This was investigated, at the start of the

experimental work (before the studies of weight gain, weight loss and caffeine administration), as described below.

12 female Wistar rats of initial weight range 209-264g (mean = 235.00 ± 4.89) were monitored for two cycles. The rats were weighed daily to detect any possible cyclical variation in weight. Body weight changes in the rat have been reported to be negative during the pro-oestrus phase but positive during the other three phases (Agadah, 1985); this being true for both lean (chow-fed) and dietary obese rats. Blood pressure measurements were recorded each morning, as described in section 2.8.3, and vaginal smears were obtained to determine the stage of the oestrous cycle (based on the technique described by Waynforth, 1980). A stainless steel loop was flamed and cooled and then dipped into water. A small sample of cells from the vaginal wall was obtained by gentle scraping and the moist scrapings smeared onto a microscope slide. The slides were allowed to air dry before staining with 0.1% methylene blue solution. The surplus solution was carefully removed from the slide by holding it under a running tap. The slides were then allowed to dry again and then examined under a microscope. The stages of the oestrous cycle were defined histologically as follows: pro-oestrus - predominantly nucleated epithelial cells, with some polymorphnuclear leucocytes, a few cornified cells and stringy mucus also present only during early pro-oestrus; oestrus - cornified epithelial cells only present, except in the very early stages, when a few irregularly-shaped nucleated cells may also be seen; metoestrus - a mixture of nucleated epithelial cells, cornified epithelial cells and leucocytes; dioestrus - mostly polymorphnuclear leucocytes present with little or no mucus.

Blood pressure measurements were made between 09.00 and 11.00 each day and the vaginal smear tests immediately after, to avoid any possible circadian variations affecting the results. The blood pressure readings obtained at different stages of the oestrous cycle were compared.

Dieting has been shown to affect the menstrual cycle of healthy young women (Pirke et al, 1985) and it is known that dietary intake varies cyclically in women according to the stage of the menstrual cycle (Dalvitt-McPhillips, 1983; Kirby & Agadah, 1986). Therefore, food intake and the menstrual cycle appear to be closely linked. An oestral rhythm in feeding, which spans a period of 4 to 5 days, has been shown in the rat (Ter Haar, 1972). Food intake, and in particular carbohydrate intake, has been shown to affect blood pressure in man (Affarah et al, 1986). It was therefore considered possible that any blood pressure variation associated with an oestral rhythm might be altered, in the dietary obese rats, with their higher energy (and higher carbohydrate) intake compared with lean rats. Therefore, a second study of blood pressure and the oestrous cycle was carried out, using the same methods as before with 6 dietary obese rats. The mean weight of these rats at the start of the study was $278.67 \pm 10.57\text{g}$ (range 246-313g). Again, two complete cycles were followed.

2.8.5 Study to investigate the effects of the sodium-rich palatable diet on plasma sodium and potassium levels.

The evidence for a role for sodium and potassium in the aetiology, prevention and treatment of hypertension has been discussed in section 1.5. Nutritional analysis of the daily food

intake of the rats (see section 3.1.1) showed that those offered the palatable diet consumed from three to five times more sodium than those given chow only. This is in agreement with earlier studies using this dietary obese rat model (Kirby, 1984). This was mainly due to the high sodium content of the most preferred food item, Rice Krispies (see Appendix 1). It is possible that the high sodium content of the diet may have affected the cardiovascular function of the dietary obese rats. In order to gain a greater understanding of the effects this higher sodium intake might have had in the dietary obese rats, plasma sodium and potassium concentrations were determined for these rats and the values compared with those for age-matched chow-fed controls. Values were also determined for previously dietary obese rats (4 weeks palatable diet) whose weight had then been reduced by the withdrawal of the palatable diet (for a further 4 weeks).

Serum sodium levels have been shown to correlate significantly with blood pressure in morbidly obese patients (Nanji & Freeman, 1985), and as the mean blood pressure values for groups of dietary obese rats have been shown to be higher than the values for lean controls (Kirby, 1984), it was considered to be of interest to investigate any differences in plasma sodium levels between these groups. The experimental procedure was as described below.

Female Wistar rats (180-220g) were randomly assigned to groups and housed six to a cage. Half of the rats received the palatable diet in addition to laboratory chow and water, and the other half received chow and water only. After 1, 2, 3, 4, 8 and 20 weeks rats were taken and blood samples removed for analysis by flame photometry (see section 2.3). After 4 weeks of receiving the palatable diet in

addition to chow plus water one group of rats then had the palatable diet withdrawn for 4 weeks and they received chow plus water only. After the 4 week withdrawal period, blood samples were removed from these rats and analysed as described earlier.

2.8.6 Methods of weight reduction

a) Withdrawal of the palatable diet

Groups of rats were offered the palatable food items (as described in 2.8.1 in addition to laboratory chow. After 4 weeks the rats who had received the palatable diet were significantly heavier than groups of age-matched controls who had received only chow. The palatable foods were then withheld from the dietary obese groups for 4 weeks. The cardiovascular parameters of heart rate and blood pressure were monitored throughout the 8 weeks of the study and at the end of the study the animals were killed and the plasma and isolated tissues studied (see sections 2.3, 2.7.2 and 2.7.3)

b) Daily administration of amphetamine (5mg/kg)

Groups of rats were offered the palatable foods in addition to laboratory chow and water for 4 weeks, after which time they were significantly heavier than chow-fed controls. Water intake was monitored daily and using the value of the mean volume of water consumed daily per rat, and the mean weight of the rats in each group, the concentration of amphetamine in the drinking water required to produce a daily dose of 5mg/kg body weight was calculated. This dose was chosen as it had been shown to produce effective weight loss in dietary obese rats in studies by Pleece (1980). The rats were then given 5mg/kg amphetamine (dissolved in the drinking water) daily for 4 weeks. The palatable diet was provided

daily as before, in addition to chow and water. Water consumption was monitored daily throughout the 8 weeks of the study, while food intake was monitored only once (during week 7) as the effects of amphetamine on food intake in dietary obese rats have been fully investigated in earlier studies (Pleece, 1980; Agadah, 1985). Body weight, heart rate and systolic blood pressure were monitored weekly throughout the study. At the end of the 8 weeks of the study the rats were killed and the isolated heart and mesenteric bed studied (see sections 2.7.2 and 2.7.3).

c) Daily administration of fenfluramine (10mg/kg)

The experimental procedure was essentially the same as for amphetamine (see section 2.8.6b)) but 10mg/kg fenfluramine (racemic mixture) was given. The dose was chosen because it had been shown to produce effective weight loss in dietary obese rats in earlier studies (Pleece, 1980; Agadah, 1985). The drug was again given in the drinking water, at a concentration such that a daily dose of 10mg/kg was taken. Fenfluramine significantly reduced fluid intake and it was therefore necessary to adjust the concentration of drug in the water accordingly. Although this effect diminished slightly as the study progressed, fluid intake still remained somewhat depressed throughout the entire study. The drug was given by direct oral dose for four consecutive days in the second week of drug treatment, to discover whether this effect on fluid intake was due to an unpalatable taste of the fenfluramine. However, fluid intake remained depressed during the period of direct oral dosing and it was therefore assumed that this was an intrinsic effect of the drug. Thereafter, the drug was then again given in the drinking water.

d) Daily administration of mazindol (4mg/kg)

The procedure was similar to that described for fenfluramine and amphetamine. The only difference being the method of administration of the drug. Mazindol was found to have a low solubility in water and it was not possible to dissolve it in the drinking water in the concentration necessary to achieve the required dose of 4mg/kg. It also proved to be insoluble in weakly acidic aqueous solutions. However, it was found to produce a colloidal suspension in a dilute (10%) solution of ethanol. The suspension was made by first suspending the mazindol in a small volume of absolute alcohol (10% final volume). This produced a 'milky' suspension. The water was then added dropwise, while the mixture was agitated constantly. The resulting suspension was not very stable and had to be shaken before the withdrawal of each dose volume, to resuspend the drug and ensure uniformity of dose. The dose of 4mg/kg was chosen because it had proved to be effective in producing weight loss in dietary obese rats in earlier studies (Pleece, 1980), and it was given in a volume of 0.25-0.30ml of the suspension. A second group of rats were treated identically to the first, but oral dosed with the vehicle only (dose volume 0.30ml), to check for any effects produced by the dosing method and/or the vehicle.

2.8.7 The effects of 25mg/kg and 50mg/kg caffeine daily, with and without the palatable diet.

The purpose of the study described below was to see whether the palatable diet (see section 2.8.1), with its high fat and sodium content would alter any cardiovascular effects of two doses of caffeine in female Wistar rats. The doses used were 25mg/kg and

50mg/kg, given dissolved in the drinking water.

The groups of animals used were as follows: lean, chow-fed control rats (L); dietary obese rats, receiving the palatable diet in addition to chow (D); lean rats (chow-fed) receiving 25mg/kg caffeine daily (LC25); dietary obese rats (palatable diet plus chow) receiving 25mg/caffeine daily (DC25); lean rats (chow-fed) receiving 50mg/kg caffeine daily (LC50); dietary obese rats (palatable diet plus chow) receiving 50mg/kg caffeine daily (DC50), (n = 12 for each group).

According to figures quoted by Yeh et al (1986), the doses of 25mg/kg and 50mg/kg in the rat are equivalent to 340mg and 680mg in a man weighing 70kg, if the conversion is based on metabolic body weight ($\text{kg}^{0.75}$) as described by Kleiber (1961). If it is assumed that an 'average' cup of coffee may contain approximately 85mg caffeine (see Appendix 3), then the doses of 25mg/kg and 50mg/kg daily in the rat are equivalent to the consumption of 4 and 8 cups of coffee daily by a 70kg man. Administration of the caffeine, with and without the palatable diet, was continued for 4 weeks. During this period weight, systolic blood pressure and heart rate were monitored each week. In addition fluid intake was recorded daily and food intake was also monitored. After 4 weeks the rats were killed and the isolated hearts and mesenteric beds studied as described earlier (see section 2.7.2 and 2.7.3).

Chapter 3 : RESULTS

CHAPTER 3 : RESULTS

3.1 THE EFFECTS OF 'AD LIBITUM' ACCESS TO A VARIED PALATABLE DIET

Whenever a period of weeks is quoted in this section, it refers to the number of weeks from the start of the study, that is, the number of weeks that the rat has received chow with or without the palatable diet.

3.1.1 Food and water intake in rats with or without the palatable diet

Food intake was monitored regularly, as described in section 2.8.2, over 16 weeks. A summary of 24-hour food intake, expressed both as grams/milligrams (g/mg) of various nutrients and energy in kilocalories (kcal) is given in Table 3.1(1). These values were calculated for the laboratory chow from the manufacturers data (see Appendix 1) and for the palatable food items from "McCance and Widdowson's: The Composition of Foods" (Paul et al, 1978) (see Appendix 1).

The energy intake for both lean and obese rats fell during the course of the study. The decrease in energy intake from Week 1 to Week 16 was approximately 25% for the lean rats and approximately 40% for the dietary obese rats. The intake of all nutrients also fell during this period, following a similar pattern.

Rats offered the palatable diet in addition to chow showed a greater energy intake throughout the 16 weeks, compared to chow-only fed lean controls, consuming approximately 150 to 200% of the energy intake of the lean controls. Carbohydrate intake was greater in the dietary obese rats than in the lean controls throughout the studies,

being 187.77% of the intake of the lean rats during week 1 and 152.14% of the lean rats during week 16. There was a fall in carbohydrate intake during the study for both lean and dietary obese rats. However, the decrease was greater in the obese rats (17.65g per rat per day in week 1 to 10.33g in week 16 for the obese rats and 9.40g in week 1 to 6.79g in week 16 for the lean rats).

The intake of fat by the dietary obese rats varied between 400 and 670% of that of the lean rats during the 16 weeks of the study. This greater relative intake of fat showed no decrease throughout the study. The intake of protein by the dietary obese rats was between 60 and 70% of that of lean controls, throughout the 16 weeks of the study period. The intake of dietary fibre was also consistently higher in the dietary obese rats compared with lean.

Sodium intake in the dietary obese rats was approximately 300 to 450% that of the lean rats throughout the 16 weeks, which was probably the major cause of the greater fluid intake by this group (see below). The greater intake of sodium was mainly due to the high sodium content of the most preferred food, Rice Krispies (see Appendix 1).

Potassium intake in the dietary obese rats was only 40 to 50% of that of the lean rats throughout the 16 weeks of the study. This was due to the fact that the laboratory chow (CRM brand) had a much higher potassium content than any of the palatable food items (see Appendix 1). Calcium intake was also greatly reduced in the dietary obese rats, at only 20 to 30% of that of the lean rats. This reduced intake persisted throughout the study and was due to the much lower calcium content of the palatable food items than that of the laboratory chow (see Appendix 1).

Table 3.1(1)

Summary of daily intake of various nutrients during the 16 weeks of study in rats offered laboratory chow with (Dietary Obese) or without (Lean) a varied palatable diet. All values expressed per rat.

	<u>Lean</u>	<u>Dietary Obese</u>	<u>Dietary obese</u> <u>as % lean</u>
<u>Week 1</u>			
Energy (kcal)	47.11	92.02	195.33
Carbohydrate (g)	9.40	17.65	187.77
Fat (g)	0.40	2.01	502.50
Protein (g)	2.99	2.13	71.24
Fibre (g)	0.59	1.07	181.36
Sodium (mg)	46.20	196.61	425.57
Potassium (mg)	123.75	59.12	47.77
Calcium (mg)	132.00	34.72	26.30
<u>Week 2</u>			
Energy (kcal)	46.87	82.39	175.78
Carbohydrate (g)	9.35	15.99	171.02
Fat (g)	0.39	1.74	446.15
Protein (g)	2.97	1.85	62.29
Fibre (g)	0.59	0.94	159.32
Sodium (mg)	45.97	190.36	414.09
Potassium (mg)	123.13	51.50	41.83
Calcium (mg)	131.33	26.87	20.46

CONTINUED OVERLEAF

Week 3

Energy (kcal)	37.89	70.82	186.91
Carbohydrate (g)	7.56	14.21	187.96
Fat (g)	0.32	1.31	409.38
Protein (g)	2.40	1.59	66.25
Fibre (g)	0.48	0.80	166.67
Sodium (mg)	34.07	173.19	508.34
Potassium (mg)	91.25	46.69	51.17
Calcium (mg)	97.33	24.12	24.78

Week 4

Energy (kcal)	45.16	81.14	179.67
Carbohydrate (g)	9.01	16.15	179.25
Fat (g)	0.38	1.54	405.26
Protein (g)	2.86	1.89	66.08
Fibre (g)	0.57	0.93	163.16
Sodium (mg)	40.60	180.75	445.20
Potassium (mg)	108.75	56.43	51.89
Calcium (mg)	116.00	33.72	29.07

Week 8

Energy (kcal)	43.42	86.25	198.64
Carbohydrate (g)	8.66	15.11	174.48
Fat (g)	0.37	2.48	670.05
Protein (g)	2.75	1.94	70.55
Fibre (g)	0.55	0.93	168.18
Sodium (mg)	42.58	185.78	436.30
Potassium (mg)	114.06	51.18	44.87
Calcium (mg)	121.67	25.44	20.91

CONTINUED OVERLEAF

Week 12

Energy (kcal)	43.68	65.42	149.77
Carbohydrate (g)	8.72	11.43	131.08
Fat (g)	0.37	1.83	494.59
Protein (g)	2.77	1.54	55.60
Fibre (g)	0.55	0.64	116.36
Sodium (mg)	42.84	148.18	345.88
Potassium (mg)	114.75	46.84	40.82
Calcium (mg)	122.40	25.05	20.47

Week 14

Energy (kcal)	36.97	61.07	165.19
Carbohydrate (g)	7.38	11.02	149.32
Fat (g)	0.31	1.60	516.13
Protein (g)	2.34	1.29	55.13
Fibre (g)	0.47	0.62	131.91
Sodium (mg)	36.26	130.36	359.51
Potassium (mg)	97.13	47.50	48.90
Calcium (mg)	103.60	29.84	28.80

Week 16

Energy (kcal)	34.03	55.67	163.59
Carbohydrate (g)	6.79	10.33	152.14
Fat (g)	0.29	1.32	455.17
Protein (g)	2.16	1.44	66.67
Fibre (g)	0.43	0.58	134.88
Sodium (mg)	33.38	130.23	390.14
Potassium (mg)	89.40	44.98	50.31
Calcium (mg)	95.36	27.69	29.04

Water intake of each group was monitored daily throughout the 16 weeks of the study and the volume consumed daily per rat calculated. The dietary obese rats consumed significantly more water than lean rats throughout the study period (see Table 3.1(ii)). The mean concentrations of various electrolytes in the tap water for the area including Bath University was obtained from the Wessex Water Authority. They are as follows : sodium (as Na) 13 p.p.m.; potassium (as K) 2.3p.p.m.; and calcium (as CaCO_3) 330 p.p.m. The dietary obese rats only consumed 2.5 to 6ml more water per rat per day than the lean controls (approximately 12.5 to 30%) and the quantities of the electrolytes contained in that small volume would not have greatly affected the overall intake and so the quantity of the electrolytes from water was omitted from the calculations.

Table 3.1(ii)

Daily water intake in rats offered laboratory chow with (Dietary Obese) or without (Lean) a varied palatable diet during 16 weeks of study. All values expressed as 'ml per rat' (mean \pm s.e.m. for 7 days of each week).

<u>Week of dietary</u> <u>treatment.</u>	<u>Lean</u>	<u>Dietary</u> <u>Obese</u>	<u>p*</u>
1	18.58 \pm 0.52	27.00 \pm 0.82	<0.001
2	20.76 \pm 0.83	27.31 \pm 1.43	<0.001
3	16.19 \pm 1.29	23.51 \pm 1.38	<0.001
4	18.51 \pm 0.76	25.70 \pm 1.22	<0.001
5	18.81 \pm 0.76	24.40 \pm 1.08	<0.001
6	19.53 \pm 0.70	26.59 \pm 1.44	<0.001
7	18.44 \pm 0.89	25.29 \pm 1.28	<0.001
8	18.11 \pm 0.55	24.60 \pm 0.73	<0.001
9	17.50 \pm 0.63	22.14 \pm 0.75	<0.001
10	18.63 \pm 0.40	22.46 \pm 0.73	<0.001
11	18.81 \pm 0.66	20.43 \pm 0.67	<0.01
12	17.91 \pm 0.28	22.70 \pm 0.97	<0.001
13	18.40 \pm 0.39	21.03 \pm 0.83	<0.001
14	18.70 \pm 0.71	20.07 \pm 0.39	<0.01
15	19.00 \pm 0.76	22.13 \pm 0.87	<0.001
16	18.68 \pm 0.52	22.21 \pm 0.79	<0.001

* Significance calculated using Student's t-test

3.1.2 Changes in weight with time for lean and dietary obese rats.

Body weight increased with time for both lean and dietary obese animals (see Table 3.1(iii) and Fig.7). After 2 weeks the dietary obese rats were significantly heavier than the lean ($p < 0.02$) and after 4 weeks this difference reached significance at $p < 0.001$ and this was maintained throughout the study.

3.1.3 Changes in minimum systolic blood pressure with time for lean and dietary obese rats.

An example of a typical trace of a 10-minute blood pressure monitoring period is shown in Fig.9. Minimum systolic blood pressure increased with time for both lean and dietary obese animals, reaching a peak in the dietary obese rats after approximately 6 weeks and in lean rats after approximately 8 weeks (see Table 3.1(iv) and Fig.8). After 4 weeks the blood pressure of the dietary obese rats was significantly higher than for the lean rats and remained higher throughout the 20 weeks of study (at $p < 0.05$ or less). The correlation coefficient (r) was calculated for weight and minimum systolic blood pressure values in both lean and obese rats after 4, 8, 10, and 20 weeks of study (see Table 3.1(v)). Significant correlation was seen for lean and obese groups combined after 8, 10, and 20 weeks. No significant correlation was seen within the lean groups and within the obese group significant correlation was seen only after 10 weeks.

Table 3.1(iii)

Weights (g) of lean and dietary obese rats over 20 weeks of study.

All values expressed as mean \pm s.e.m. for group, n=12 unless otherwise stated.

<u>Number of weeks of</u> <u>study completed.</u>	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
0	211.4 \pm 3.1	209.2 \pm 3.6	n.s.
1	226.3 \pm 3.2	224.8 \pm 4.2	n.s.
2	230.4 \pm 3.6	246.9 \pm 4.9	<0.02
3	238.8 \pm 4.2	258.9 \pm 5.5	<0.01
4	243.0 \pm 4.1	275.3 \pm 6.8	<0.001
5	249.5 \pm 4.8	283.7 \pm 7.3	<0.001
6	254.3 \pm 5.0	294.7 \pm 7.8	<0.001
7	258.8 \pm 5.6	308.3 \pm 8.7	<0.001
8	265.3 \pm 5.9	316.4 \pm 9.9	<0.001
10	267.8 \pm 6.7	318.7 \pm 10.6	<0.001
20	304.0 \pm 10.3(n=10)	389.9 \pm 14.7(n=5)	<0.001

* Significance calculated using Student's t-test

n.s.= not significant at $p < 0.05$

Fig.7
Change in weight with time for lean and dietary obese rats (mean \pm s.e.m., n=12 unless stated).

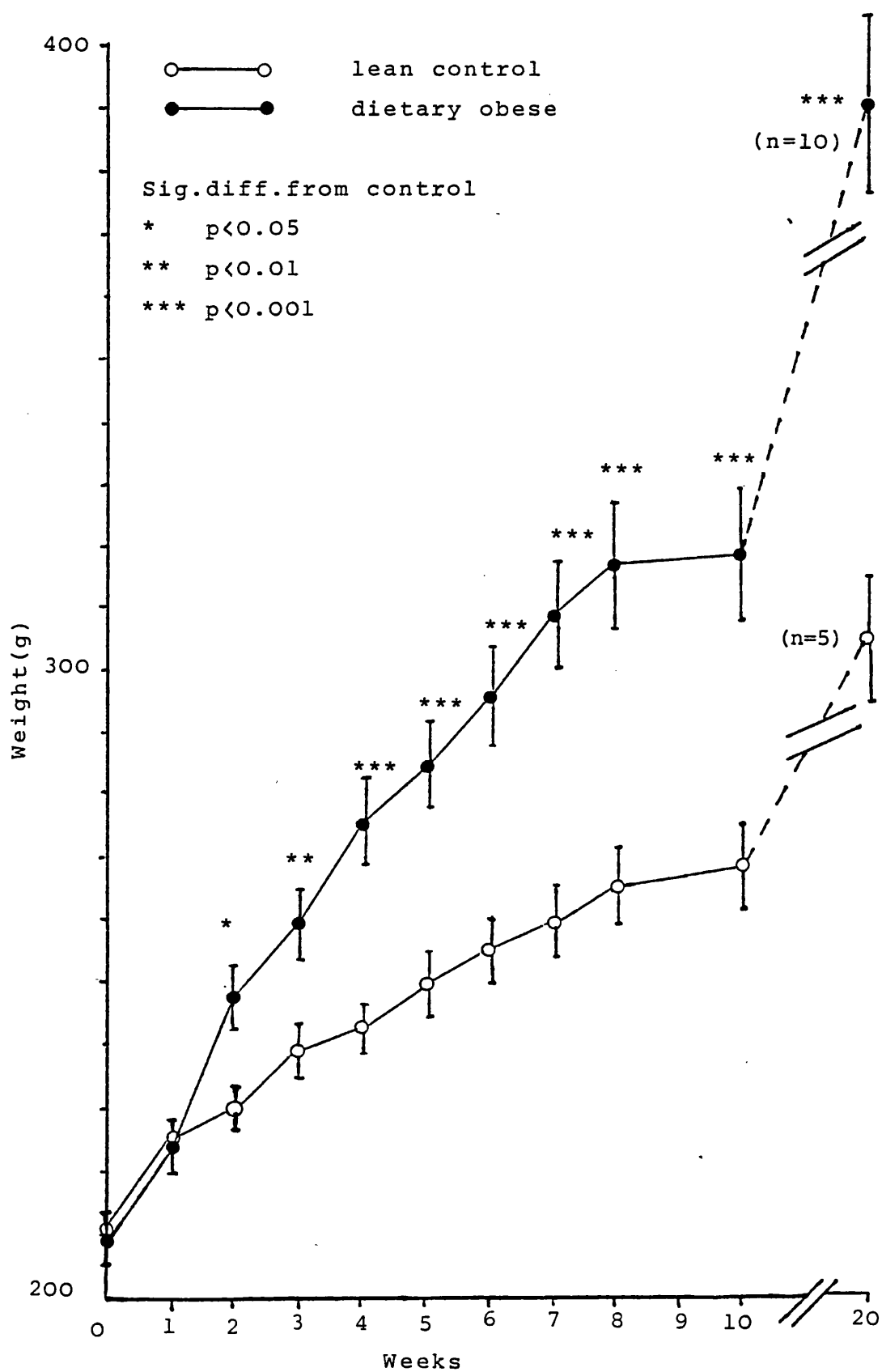


Table 3.1(iv)

Systolic blood pressure (mmHg) of lean and dietary obese rats over 20 weeks of study. All values expressed as mean \pm s.e.m. for group, n=12 unless otherwise stated.

<u>Number of weeks of</u> <u>study completed.</u>	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
0	105.9 \pm 1.2	107.0 \pm 3.5	n.s.
1	107.7 \pm 1.8	108.2 \pm 2.7	n.s.
2	105.7 \pm 1.6	115.3 \pm 3.6	<0.05
3	110.5 \pm 3.8	118.1 \pm 3.3	n.s.
4	114.3 \pm 3.3	125.4 \pm 3.4	<0.05
5	122.9 \pm 4.4	143.0 \pm 3.4	<0.005
6	137.9 \pm 3.5	156.1 \pm 3.3	<0.005
8	140.3 \pm 3.2	152.4 \pm 3.2	<0.05
10	130.3 \pm 3.0	147.3 \pm 4.7	<0.01
20	130.8 \pm 2.9(n=10)	144.3 \pm 2.4(n=5)	<0.05

* Significance calculated using Student's t-test

n.s.= not significant at P<0.05

Fig.8

Change in blood pressure with time for control
and dietary obese rats.

(mean \pm s.e.m., n=12 unless stated)

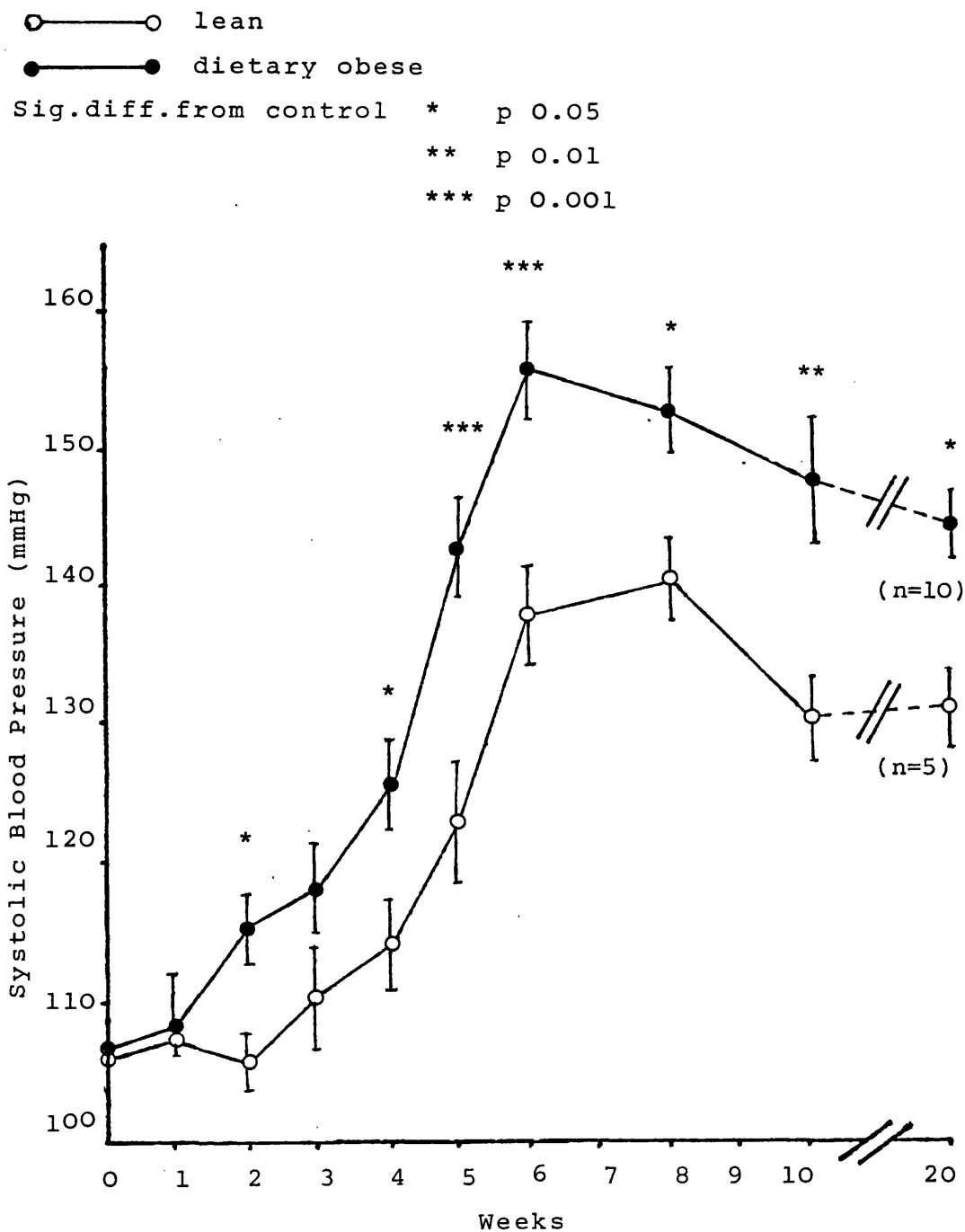


Fig.9 Trace showing tail-cuff blood pressure measurements for one rat during one 10 minute recording period. The means of the "cut-off" and "restart" values were used to obtain the systolic blood pressure reading each time.

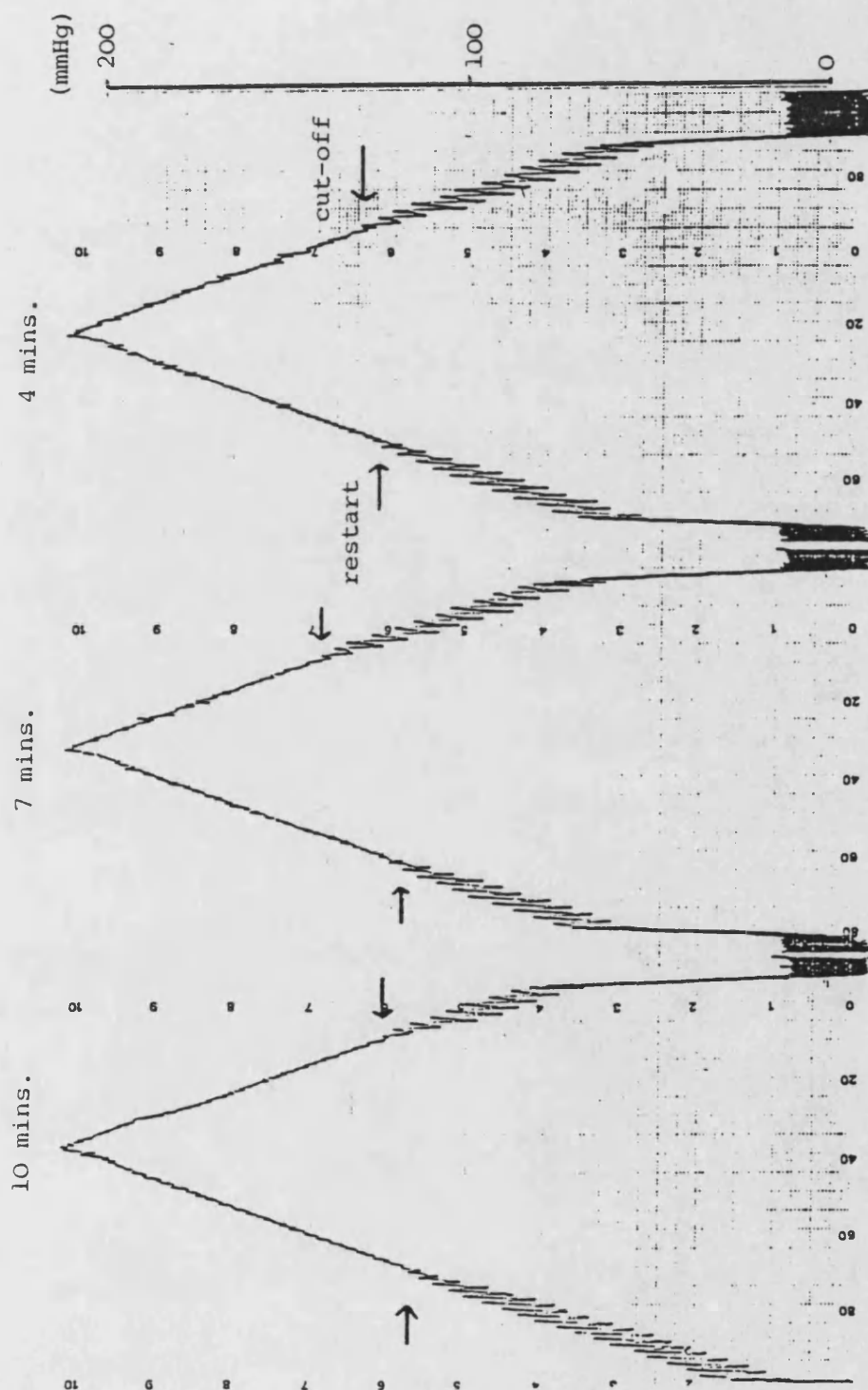


Table 3.1(v)

Correlation coefficient (r) for weight (g) and systolic blood pressure (mmHg) of lean and dietary obese rats throughout 20 weeks of study.

	<u>After</u> <u>4 weeks</u>	<u>After</u> <u>8 weeks</u>	<u>After</u> <u>10 weeks</u>	<u>After</u> <u>20 weeks</u>
Lean	-0.1000 (n=12)	0.2078 (n=12)	0.4462 (n=12)	0.8537 (n=5)
Dietary Obese	-0.0871 (n=12)	0.5311 (n=12)	0.8819* (n=12)	0.4057 (n=10)
Lean + Obese	0.2294 (n=24)	0.5925** (n=24)	0.8348*** (n=24)	0.6549** (n=15)

* significant correlation $p < 0.05$

** significant correlation $p < 0.01$

*** significant correlation $p < 0.001$

Table 3.1(vi)

Correlation coefficient (r) for weight (g) and heart rate (beats per minute) of lean and dietary obese rats throughout 20 weeks of study.

	<u>After</u> <u>4 weeks</u>	<u>After</u> <u>8 weeks</u>	<u>After</u> <u>10 weeks</u>	<u>After</u> <u>20 weeks</u>
Lean	0.3990 (n=12)	0.1874 (n=12)	0.1517 (n=12)	0.3507 (n=5)
Dietary Obese	-0.0719 (n=12)	-0.4845 (n=12)	-0.4906 (n=12)	0.3644 (n=10)
Lean + Obese	0.3639 (n=24)	0.2192 (n=24)	0.1141 (n=24)	0.4065 (n=15)

No significant values.

3.1.4 Changes in heart rate with time for lean and dietary obese rats. (see Table 3.1(vii))

At the start of the study heart rates were similar in the two groups. However, after 4 weeks the heart rates of the dietary obese rats were significantly faster than those of the lean controls ($p < 0.001$). This faster heart rate in the dietary obese rats was observed throughout the rest of the study, though after 20 weeks the difference was not significant. However, the numbers in the lean control group were small at this point ($n=5$). The correlation coefficient (r) was calculated for weight and heart rate for lean and obese groups after 4, 8, 10, and 20 weeks (see Table 3.1(vi)). No significant correlation was found between weight and heart rate for the groups, either separately or combined.

Table 3.1(vii)

Heart rates (beats per minute) of lean and dietary obese rats throughout 20 weeks of study. All values expressed as mean \pm s.e.m. for group.

<u>Number of weeks</u> <u>of study completed</u>	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
0	380.67 \pm 10.21 (n=12)	372.04 \pm 6.68 (n=12)	n.s.
4	379.33 \pm 8.14 (n=12)	399.04 \pm 7.22 (n=12)	<0.001
8	386.71 \pm 10.59 (n=12)	415.50 \pm 7.52 (n=12)	<0.001
10	416.46 \pm 6.66 (n=12)	437.71 \pm 9.49 (n=12)	<0.001
20	378.80 \pm 23.42 (n=5)	403.85 \pm 10.65 (n=10)	n.s.

* Significance calculated using Student's t-test

3.1.5 'In vitro' studies: the isolated perfused heart

The isolated perfused heart preparation was studied from lean and dietary obese rats after 4 and 8 weeks.

a) Responses of isolated perfused hearts, after 4 weeks, to doses of noradrenaline

No significant differences in the 'in vitro' beating rate were seen between hearts from lean and dietary obese groups, either for baseline beating rate or for drug-induced increases in beating rate (see Table 3.1(viii)a)). This is in contrast to the small but significant increase in heart rate found in the conscious whole animal studies (see section 3.1.4). Furthermore, there was no significant difference between baseline contractility of hearts from lean and obese groups (see Table 3.1(viii)b)). However, there was a significantly greater contractility response (% baseline contractility) to all three doses of noradrenaline in hearts from dietary obese rats compared to those from lean, significant $p < 0.001$ for responses to 5×10^{-11} mole noradrenaline and $p < 0.01$ for 5×10^{-10} mole and 5×10^{-9} mole.

After each dose of noradrenaline the number of arrhythmias (ectopic beats and missed beats) which occurred within the first three minutes was recorded. This data, presented in Table 3.1(x)a), was analysed using the non-parametric Mann-Whitney U-test (a non-parametric test was required due to the distribution of the data points). No significant differences were found between the groups after any of the doses of noradrenaline.

b) Responses of isolated perfused hearts, after 8 weeks, to doses of noradrenaline

There were no significant differences in beating rates, either

baseline values or drug-induced responses, between hearts from lean and obese rats (see Table 3.1(ix)a)), again highlighting a difference between 'in vitro' and 'in vivo' data, a significant elevation in heart rate having been found in conscious dietary obese rats compared with lean controls at 8 weeks. In contrast to the data obtained after 4 weeks with or without the palatable diet, where no significant difference was detected, baseline contractility was significantly smaller in hearts from dietary obese rats than in those from the lean controls ($p < 0.001$) (see Table 3.1(ix)b) for contractility data). Although the response (%baseline contractility) to the low dose (5×10^{-11} mole) of noradrenaline was similar for hearts from lean and obese rats, the responses to 5×10^{-10} mole and 5×10^{-9} mole were significantly greater for hearts from dietary obese rats than for those from lean controls (both $p < 0.001$), showing a similar pattern to that seen in data obtained after 4 weeks (see above).

Again (as for the data obtained after 4 weeks) no significant differences were found between the number of arrhythmias which occurred in hearts from lean and obese rats after the three doses of noradrenaline.

c) Responses of isolated perfused hearts, after 4 and 8 weeks, to doses of acetylcholine

The duration of cardiac arrest and recovery time were dose-dependent in hearts from both lean and obese rats at both sampling times (see Table 3.1(xi)). Considering first the duration of cardiac arrest, it can be seen that at 4 weeks there was little difference between the groups, although a small but significant increase in response to the lower dose of acetylcholine was seen in hearts from the obese animals. At 8 weeks the data indicated a trend

for increased periods of cardiac arrest following acetylcholine doses in hearts from obese rats. However, this did not reach significance. The results for the recovery period after acetylcholine-induced cardiac arrest appear to alter with time, thus at the 4 week stage hearts from the obese rats recovered more rapidly than those from lean rats. This reached significance only with the high dose of acetylcholine. In contrast, after 8 weeks of study there were significantly prolonged recovery times with both doses of acetylcholine in hearts from obese animals when compared with hearts from lean controls. In addition, the recovery times seen at 8 weeks were significantly greater than those at 4 weeks for obese rats ($p < 0.001$ for both doses of acetylcholine), while the recovery period of hearts from the lean groups did not show any alteration with time.

3.1.6 'In vitro' studies: the aortic spiral strip

The responses to doses of noradrenaline of aortic spiral strips from lean ($n=5$) and obese ($n=5$) rats after 8 weeks were studied. The log dose/response curve is shown in Fig.10. The EC_{50} (that is, the concentration required to produce 50% maximal response) of noradrenaline was significantly greater for tissues from obese rats ($8.03 \pm 1.52 \times 10^{-9} M$) than for tissues from the lean group ($2.93 \pm 1.0 \times 10^{-9} M$), $p < 0.02$, indicating a relative insensitivity of tissues from the dietary obese animals.

Table 3.1(viii)

The responses of isolated perfused hearts from lean and dietary obese rats, after 4 weeks of study, to bolus injections of noradrenaline.

(All values expressed as mean \pm s.e.m. for group).

a) Beating rate /beats per minute

	<u>Lean (n=9)</u>	<u>Dietary Obese(n=9)</u>	<u>p*</u>
Baseline beating rate	271.67 \pm 6.29	262.22 \pm 10.35	n.s.
Increase in beating rate induced by dose of noradrenaline.			
5 x 10 ⁻¹¹ mole	17.78 \pm 2.37	20.56 \pm 6.94	n.s.
5 x 10 ⁻¹⁰ mole	58.33 \pm 6.12	53.33 \pm 14.84	n.s.
5 x 10 ⁻⁹ mole	88.33 \pm 11.02	78.89 \pm 13.74	n.s.

b) Contractility (against 2g tension)/g

	<u>Lean (n=9)</u>	<u>Dietary Obese(n=9)</u>	<u>p*</u>
Baseline contractility	6.56 \pm 1.23	6.17 \pm 0.59	n.s.
Response to dose of noradrenaline (% baseline contractility)			
5 x 10 ⁻¹¹ mole	142.57 \pm 6.85	175.59 \pm 8.46	<0.001
5 x 10 ⁻¹⁰ mole	192.43 \pm 8.19	231.09 \pm 20.96	<0.01
5 x 10 ⁻⁹ mole	211.50 \pm 10.87	255.36 \pm 21.13	<0.01

* Significance calculated using Student's t-test

Table 3.1(1x)

The responses of isolated perfused hearts from lean and dietary obese rats, after 8 weeks of study, to bolus injections of noradrenaline. (All values expressed as mean \pm s.e.m. for group).

a) Beating rate /beats per minute

	<u>Lean</u> (n=9)	<u>Dietary Obese</u> (n=11)	<u>p*</u>
Baseline beating rate	281.11 \pm 7.85	275.45 \pm 13.31	n.s.
Increase in beating rate induced by dose of noradrenaline.			
5 x 10 ⁻¹¹ mole	10.00 \pm 3.63	19.09 \pm 5.59	n.s.
5 x 10 ⁻¹⁰ mole	47.78 \pm 13.13	63.18 \pm 12.55	n.s.
5 x 10 ⁻⁹ mole	102.78 \pm 10.51	113.18 \pm 13.40	n.s.

b) Contractility (against 2g tension)/g

	<u>Lean</u> (n=9)	<u>Dietary Obese</u> (n=11)	<u>p*</u>
Baseline contractility	11.78 \pm 1.02	7.93 \pm 0.64	<0.001
Response to dose of noradrenaline (% baseline contractility)			
5 x 10 ⁻¹¹ mole	144.62 \pm 4.21	151.60 \pm 6.17	n.s.
5 x 10 ⁻¹⁰ mole	153.29 \pm 7.11	176.09 \pm 9.68	<0.001
5 x 10 ⁻⁹ mole	170.88 \pm 8.76	195.63 \pm 9.99	<0.001

* Significance calculated using Student's t-test

Analysis of covariance on log.dose of noradrenaline vs response showed no significant difference between the slopes of the lines for hearts from lean and obese rats, both when response was expressed as absolute contractility and also when it was expressed as % baseline contractility (F=0.577 and F=0.952, respectively, both D.F. 1,56 , not significant).

Table 3.1(x)

The number of arrhythmias (ectopic beats and missed beats) occurring in the isolated perfused hearts from lean and obese rats after three doses of noradrenaline (values for each preparation shown).

a) After 4 weeks of study

	<u>Lean</u> (n=9)	<u>Dietary Obese</u> (n=9)
5×10^{-11} mole	1,1,1,0,36,0,1,0,0	0,0,0,0,0,0,0,0,0
5×10^{-10} mole	0,2,0,1,0,9,2,0,0	1,1,2,0,3,1,22,0,0,
5×10^{-9} mole	2,0,0,1,2,16,2,0,0	143,2,1,0,2,2,0,0,0

b) After 8 weeks of study

	<u>Lean</u> (n=9)	<u>Dietary Obese</u> (n=11)
5×10^{-11} mole	0,8,0,0,0,0,2,0,0	0,0,0,0,0,0,0,1,0,0,0
5×10^{-10} mole	0,3,1,4,14,0,6,6,0	0,0,1,0,0,0,2,10,1,0,0
5×10^{-9} mole	10,0,0,63,6,0,7,0,35	0,0,1,1,0,0,0,1,2,0,0

No significant differences were found between hearts from lean and obese groups (using non-parametric, Mann-Whitney U-test).

Table 3.1(xi)

The responses of isolated perfused hearts from lean and dietary obese rats (after 4 and 8 weeks) to two doses of acetylcholine. Values expressed as mean \pm sem.

a) Duration of cardiac arrest induced by dose of acetylcholine (seconds).

	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
<u>After 4 weeks</u>			
5 x 10 ⁻⁹ mole	2.30 \pm 0.73(n=9)	3.87 \pm 0.83(n=9)	<0.05
5 x 10 ⁻⁸ mole	8.16 \pm 1.16(n=9)	7.78 \pm 0.82(n=9)	n.s.
<u>After 8 weeks</u>			
5 x 10 ⁻⁹ mole	3.84 \pm 0.94(n=9)	5.60 \pm 0.90(n=11)	n.s
5 x 10 ⁻⁸ mole	8.56 \pm 1.38(n=9)	10.87 \pm 1.19(n=11)	n.s.

b) Time to recover regular beating rhythm after dose of acetlycholine (seconds).

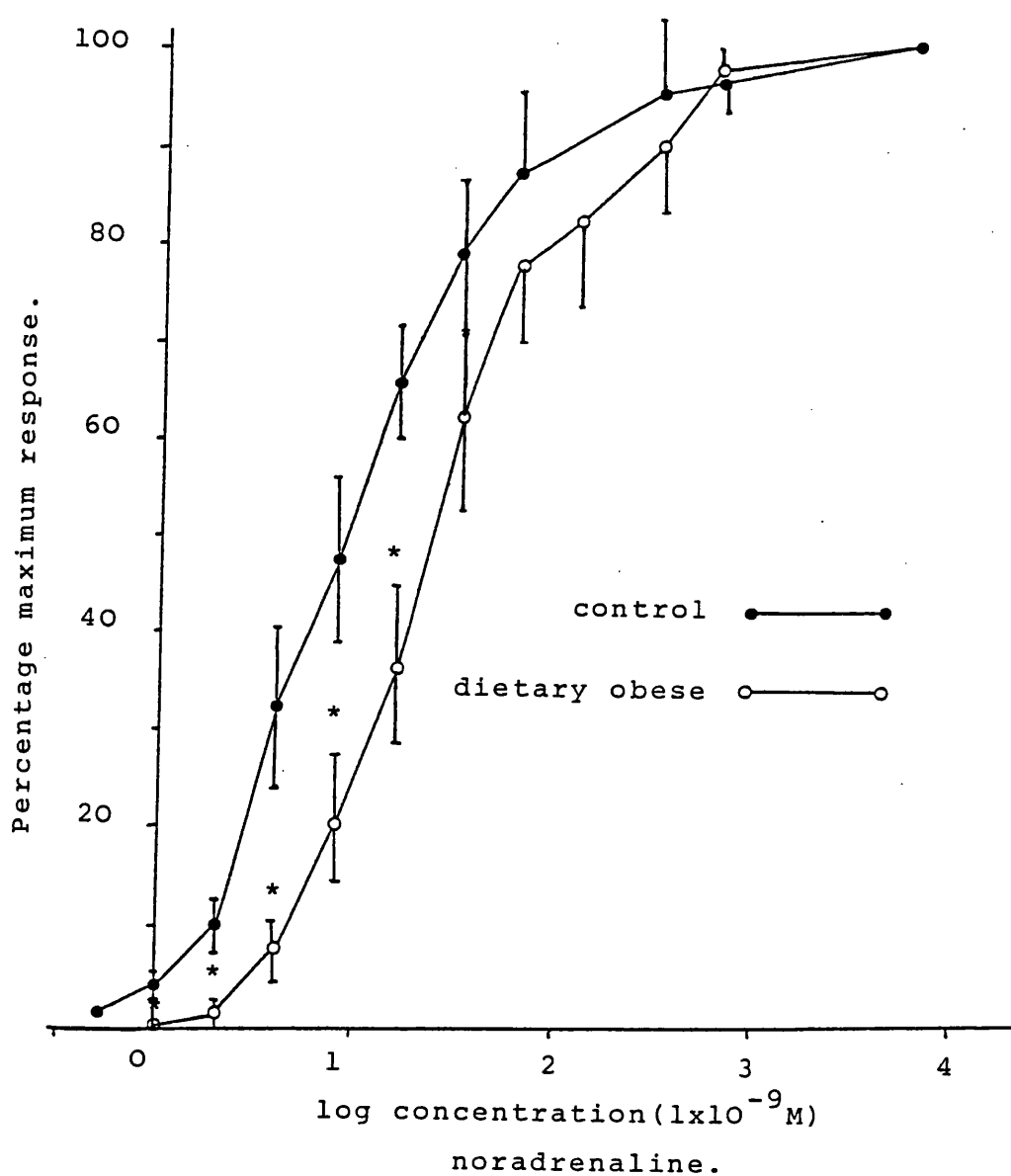
	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
<u>After 4 weeks</u>			
5 x 10 ⁻⁹ mole	12.30 \pm 2.65(n=9)	11.61 \pm 2.67(n=9)	n.s.
5 x 10 ⁻⁸ mole	38.47 \pm 2.97(n=9)	31.20 \pm 2.47(n=9)	<0.01
<u>After 8 weeks</u>			
5 x 10 ⁻⁹ mole	11.12 \pm 2.30(n=9)	16.63 \pm 1.29(n=11)	<0.001
5 x 10 ⁻⁸ mole	37.66 \pm 2.27(n=9)	46.11 \pm 4.13(n=11)	<0.005

* Significance calculated using Student's t-test

Fig.10

Effect of noradrenaline on isolated aorta spiral preparations from control and dietary obese rats.
(mean \pm s.e.m., n=5 for both groups).

* Sig.diff.from control $p < 0.05$



3.1.7 'In vitro' studies:the isolated perfused mesenteric vasculature

a) Responses to noradrenaline

Dose-response studies were carried out on the isolated perfused mesenteric vascular bed from lean and dietary obese rats after 4, 8, 10 and 20 weeks. Fig.11 shows a typical dose-response trace. The maximal response to noradrenaline (expressed as increase in perfusion pressure, mmHg) was significantly greater for tissues from dietary obese rats compared with lean after 8 weeks (171.7 ± 3.4 and 156.9 ± 4.3), $p < 0.001$, 10 weeks (235.0 ± 8.3 and 184.6 ± 20.0), $p < 0.01$ and 20 weeks (223.0 ± 3.8 and 194.6 ± 12.9), $p < 0.001$ (see Fig.12). Also there was an increase in maximal perfusion pressure response for tissues from both lean and obese rats with time, for lean rats throughout the 20 weeks of study and for obese rats up to 10 weeks.

The ED_{50} (that is, the dose required to produce 50% maximal response) values varied considerably with time though the differences between the lean and obese groups showed no specific trend (see Table 3.1(x11)). For example, at 10 weeks the sensitivity of the mesenteric bed to noradrenaline appeared to fall to approximately 25% of its value at 8 weeks for both groups. At 4 weeks the dietary obese group had a significantly higher ED_{50} (lower sensitivity) than lean, while at 8 and 10 weeks there was no apparent difference between the ED_{50} values for the two groups. However, at 20 weeks the ED_{50} for the dietary obese rats was significantly lower than for lean controls.

Fig. 11 Dose-response trace of the isolated perfused mesenteric vasculature of the rat to noradrenaline.

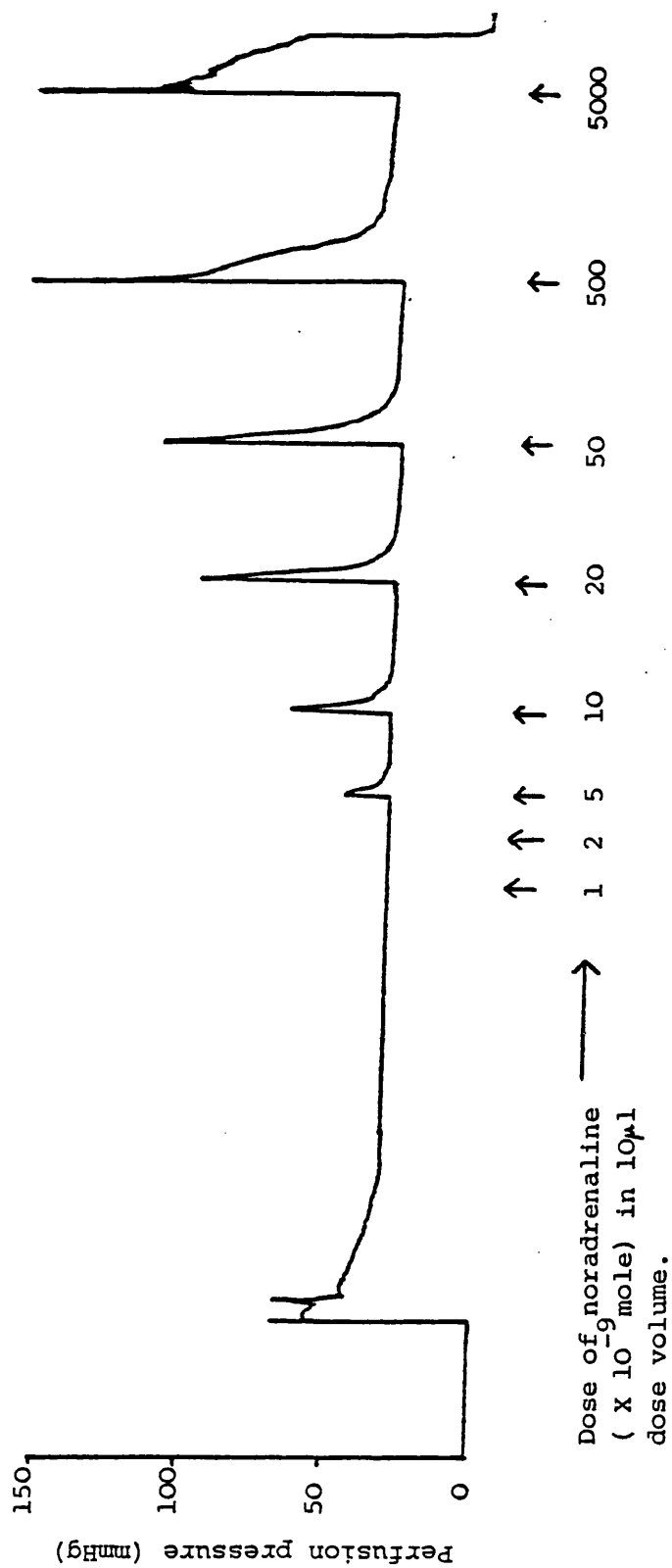


Fig. 12

Effect of noradrenaline on perfusion pressure in the isolated mesenteric vasculature taken from control and dietary obese rats.

(mean \pm s.e.m.)

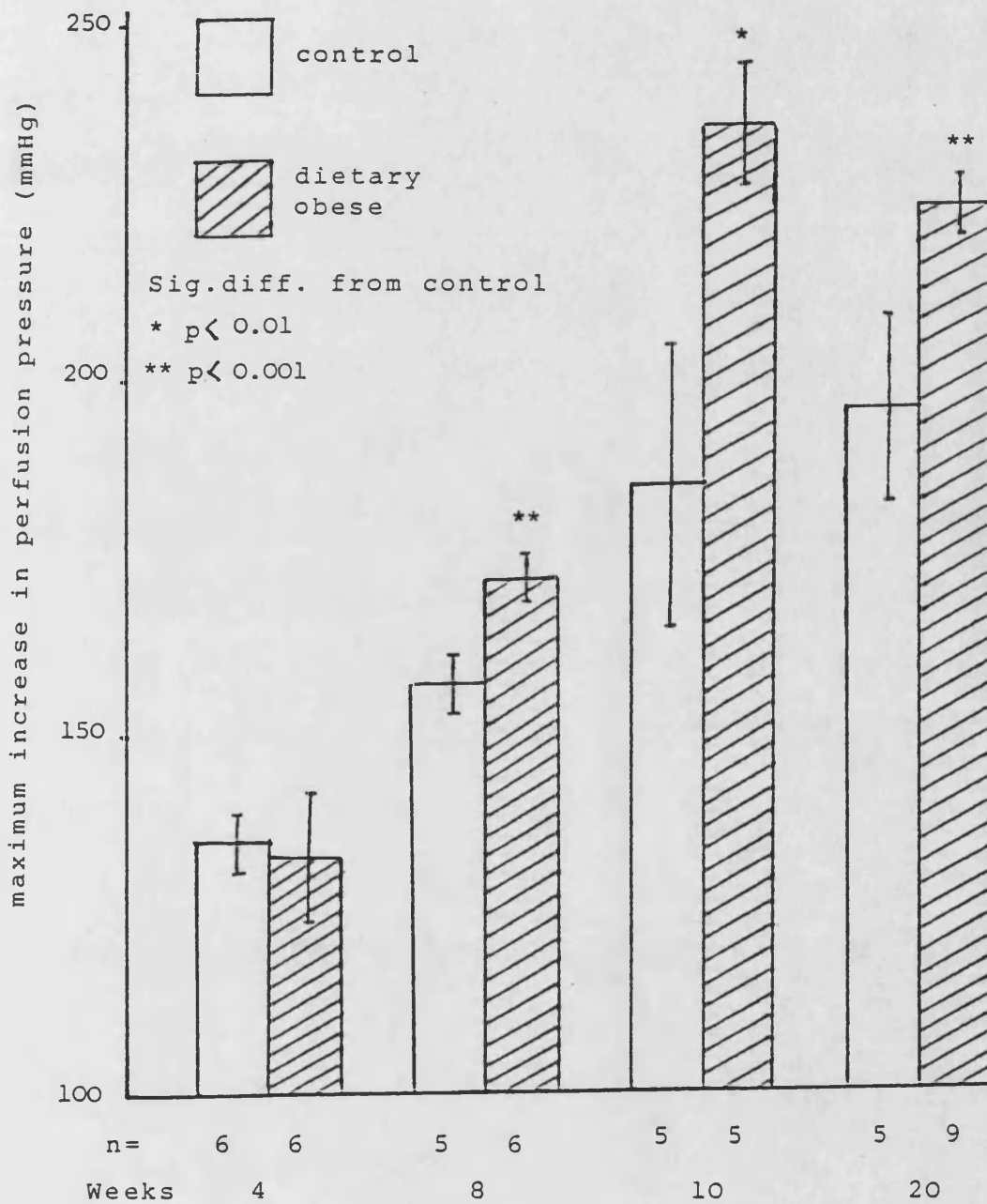


Table 3.1(xii)

The dose of noradrenaline required to produce 50% maximal response in the isolated perfused mesenteric vasculature (ED_{50}) from lean and dietary obese rats after 4,8,10 and 20 weeks of study. All values expressed as mean \pm s.e.m. for group. Units are ' $\times 10^{-10}$ mole'.

Number of weeks of

<u>study completed</u>	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
4	27.8 \pm 5.9 (n=6)	45.7 \pm 4.0 (n=6)	<0.05
8	32.1 \pm 6.0 (n=5)	27.9 \pm 2.9 (n=5)	n.s.
10	119.0 \pm 17.4 (n=5)	128.9 \pm 16.6 (n=5)	n.s.
20	168.1 \pm 29.0 (n=5)	82.5 \pm 17.1 (n=9)	<0.05

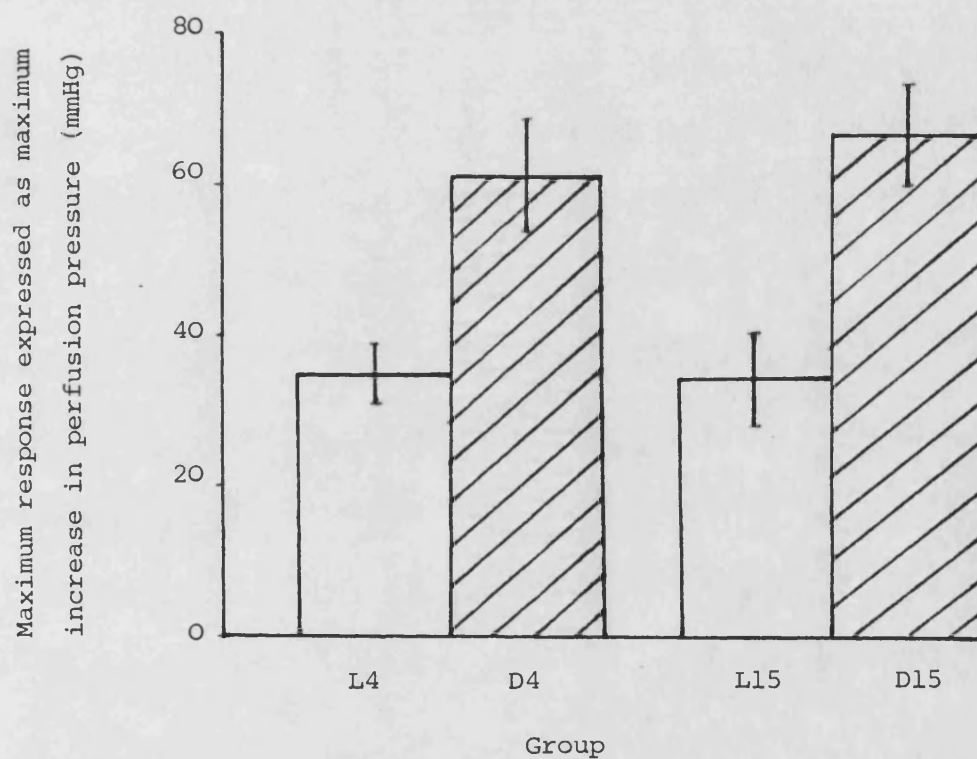
* Significance calculated using Student's t-test

b) Responses to 5-hydroxytryptamine (5-HT)

Dose-response studies to 5-HT were carried out in the isolated perfused mesenteric vasculature from lean and dietary obese rats after 4 and 15 weeks. Considering first the 4 week data; the ED_{50} (as defined earlier for noradrenaline) for tissues from lean rats was significantly greater than for tissues from dietary obese rats ($4.44 \pm 0.56 \times 10^{-9}$ mole and $3.24 \pm 0.29 \times 10^{-9}$ mole), $p < 0.005$. The maximal response, expressed as maximum increase in perfusion pressure (mmHg) to 5-HT was significantly greater for tissues from dietary obese rats compared with lean controls (61.64 ± 7.45 and 35.15 ± 4.09), $p < 0.001$, (see Fig.13). After 15 weeks, the ED_{50} for tissues from lean rats was again greater than that of tissues from obese rats ($4.90 \pm 0.44 \times 10^{-9}$ mole and $4.22 \pm 0.52 \times 10^{-9}$ mole), though this difference was not significant. As for the 4 week data, the maximum increase in perfusion pressure (mmHg) induced by 5-HT was again greater for the mesenteric vascular beds from obese rats (66.88 ± 6.50) than those from lean rats (34.60 ± 6.10), $p < 0.005$ (see Fig.13).

In contrast to the results found with noradrenaline (see section 3.1.7a) there was no time-related change in the maximum perfusion pressure increase response to 5-HT. However, a small but significant increase in the ED_{50} of 5-HT was seen in the mesenteric beds from obese animals with time ($3.24 \pm 0.29 \times 10^{-9}$ mole after 4 weeks and $4.22 \pm 0.52 \times 10^{-9}$ mole after 15 weeks), $p < 0.05$. A smaller increase was seen in tissues from lean rats ($4.44 \pm 0.56 \times 10^{-9}$ mole after 4 weeks and $4.90 \pm 0.44 \times 10^{-9}$ mole after 15 weeks), which did not reach significance.

Fig. 13 Maximum response to 5-hydroxytryptamine of the isolated perfused mesenteric vasculature from lean and dietary obese rats after 4 and 15 weeks dietary treatment. All values expressed as mean \pm s.e.m.



Key

L4 = Lean, 4 weeks (n=8)

D4 = Dietary obese, 4 weeks (n=8)

L15 = Lean, 15 weeks (n=10)

D15 = Dietary obese, 15 weeks (n=11)

c) Responses to acetylcholine after pre-contraction with phenylephrine

The isolated mesenteric vasculature was studied from lean and obese rats after 4 and 10 weeks. Considering first the 4 week data; a concentration of 10^{-3} M phenylephrine (dissolved in Kreb's solution) was found to produce an increase in vascular tone, bringing the perfusion pressure to a steady level (88.55 ± 11.09 mmHg for tissues from lean rats ($n=6$) and 71.55 ± 11.04 mmHg for tissues from obese animals ($n=8$)). These values were not significantly different. The maximum reduction in perfusion pressure (mmHg) induced by acetylcholine was 57.82 ± 12.10 for tissues from lean rats and 42.18 ± 9.64 for tissues from obese rats. There was no significant difference between the responses of the two groups, though the maximal responses for tissues from obese rats tended to be smaller than those for tissues from lean controls. The ED_{50} of acetylcholine was significantly greater for mesenteric beds from obese rats ($11.66 \pm 3.34 \times 10^{-11}$ mole) than for those tissues from lean rats ($5.95 \pm 0.86 \times 10^{-11}$ mole), $p < 0.05$.

After 10 weeks, perfusion with phenylephrine produced a greater vasoconstriction in the mesenteric beds from both lean ($n=8$) and obese ($n=10$) rats, compared to the response at 4 weeks. Thus, a tenfold reduction in the concentration of phenylephrine was used for these tissues. Phenylephrine (10^{-4} M) produced a perfusion pressure (mmHg) of 96.75 ± 10.14 for tissues from lean rats and 103.65 ± 13.21 for tissues from obese rats. Despite the reduced concentration of phenylephrine used, responses of tissues from the dietary obese rats were significantly greater than the 4 week values ($p < 0.005$). In addition, there was an elevated response for tissues from lean rats

compared to 4 week data, though this difference did not reach significance. As for the 4 week data, there was no significant difference between the perfusion pressures induced by phenylephrine in tissues from lean and obese animals.

The maximum decrease in perfusion pressure (mmHg) induced by acetylcholine was 73.69 ± 10.89 for tissues from the lean rats and 65.90 ± 11.24 for tissues from the dietary obese group. These values were not significantly different. Also there was no significant difference between the ED_{50} of acetylcholine for the two groups, the value for tissues from lean rats being $8.49 \pm 1.85 \times 10^{-11}$ mole and the value for tissues from obese rats $10.09 \pm 3.55 \times 10^{-11}$ mole.

d) Responses to changes in the concentrations of potassium and calcium in the perfusing Kreb's solution.

Fig.14 shows a typical recording trace using the following experimental procedure. Each tissue was first perfused for 30 minutes with 'normal' Kreb's solution (see section 2.2.1a)) and the peak pressure (Peak Pressure 1), the plateau pressure (Plateau Pressure 1) (both in mmHg) and time (Time to Plateau 1) (in minutes) were recorded. The tissue was then perfused for a further 30 minutes with Kreb's solution containing 65mM potassium (K^+) (see section 2.2.1c)) and the peak pressure (Peak Pressure 2), plateau pressure (Plateau Pressure 2) and the time to plateau pressure (Time to Plateau 2) recorded. The tissue was then perfused with Kreb's solution containing 65mM K^+ and 3mM calcium (Ca^{2+}) (see section 2.2.1d)) for a final 30 minutes and the plateau pressure (Plateau Pressure 3) and time to reach this plateau (Time to Plateau 3) recorded. The plateau pressure was also the peak pressure for this solution as no initial

steep rise in pressure occurred.

There were no significant differences between tissues from lean and obese rats, after 4 weeks, for any of the responses recorded (see Table 3.1(xiii)). After 8 weeks, there was a small but significant elevation in the plateau pressure obtained with the 'normal' Kreb's solution in tissues from lean rats (30.59 ± 1.10 mmHg) compared to tissues from the dietary obese rats (27.11 ± 0.92 mmHg), $p < 0.001$. However, the plateau pressure (mmHg) obtained with the 65mM K^+ Kreb's solution was higher in tissues from obese rats than in those from lean rats (55.00 ± 4.47 and 42.60 ± 1.47), $p < 0.001$. Plateau Pressure 3 (mmHg) was again higher in tissues from dietary obese rats compared with those from the lean group (71.32 ± 6.98 and 55.92 ± 2.91), $p < 0.005$. Thus, the mesenteric vasculature from these dietary obese rats appears to be more sensitive to increases in concentrations of potassium and calcium in the perfusate than tissues from age-matched lean rats. This increased sensitivity is also reflected in the greater value of peak pressure obtained with the 65mM K^+ Kreb's solution (Peak Pressure 2) in the mesenteric vasculature from the obese group (94.87 ± 7.69 mmHg) compared with that obtained in tissues from lean rats (63.49 ± 3.39 mmHg), $p < 0.001$.

The time to reach plateau pressure with 'normal' Kreb's solution was significantly greater for the mesenteric vasculature from obese rats (17.04 ± 0.88 minutes) than for tissues from lean rats (12.46 ± 0.89 minutes), $p < 0.001$. A similar pattern was seen for the time to reach Plateau Pressure 2, 16.27 ± 1.53 minutes for tissues from obese rats and 10.25 ± 1.09 for tissues from lean rats, $p < 0.001$. The time to reach Plateau Pressure 3 was significantly less for tissues from obese rats (21.82 ± 1.92) than for those from lean rats ($27.00 \pm$

0.95), $p < 0.001$. It is of interest to note that this latter time was the time to reach maximum pressure, whereas the other two time periods referred to the times to reach the minimum pressure levels. Therefore the tissues from the obese rats attained the high plateau (Plateau Pressure 3) more rapidly but took longer to reach the low plateaux, than the tissues from the lean rats.

Fig. 14 Trace showing the response of the isolated perfused mesenteric vasculature of the rat to perfusion with Krebs' solution containing different concentrations of calcium and potassium. (see section 3.1.7)

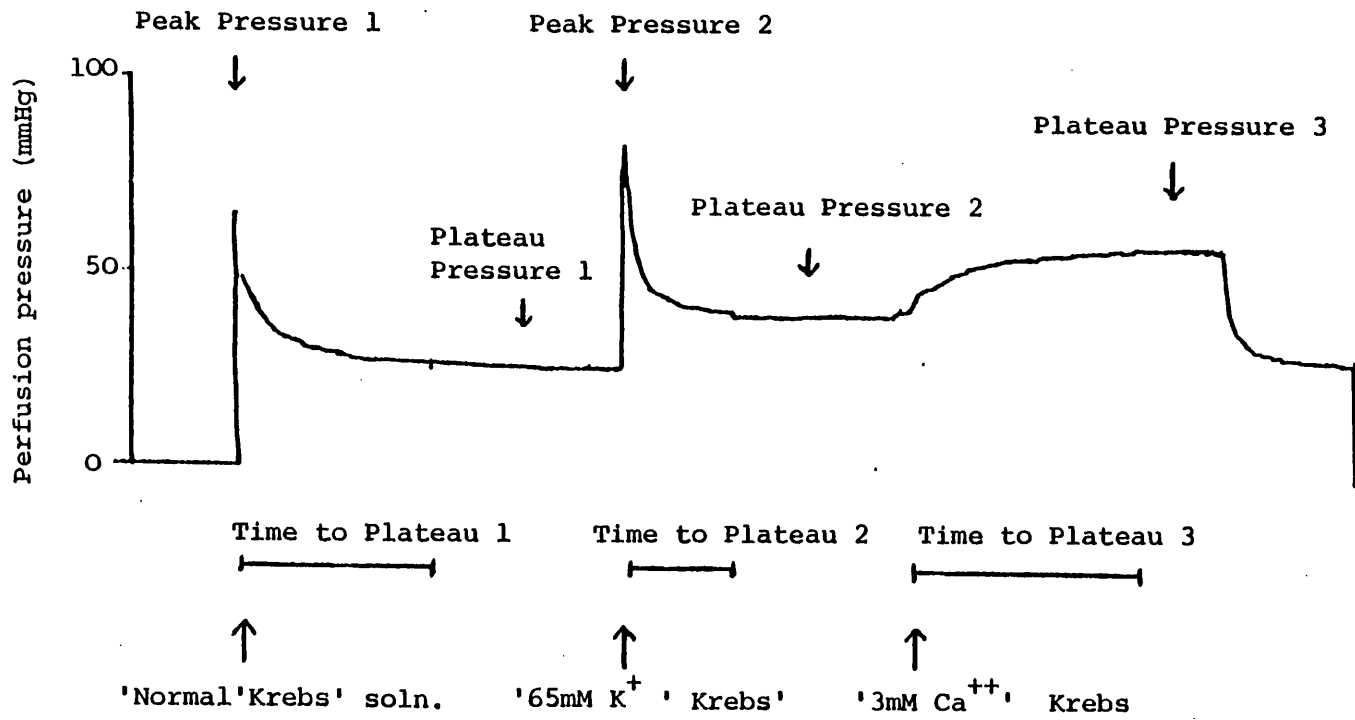


Table 3.1(xiii)

Responses of the isolated perfused mesenteric vasculature from lean and dietary obese rats, after 4 weeks, to changes in the concentrations of potassium and calcium in the perfusing Kreb's solution (see Fig.14 for specimen trace). All values shown as mean \pm s.e.m.. See section 2.2.1 for details of the different Kreb's solutions used.

		<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
<u>Peak pressure (mmHg)</u>				
('Normal' Kreb's) 1		61.73 \pm 5.85	67.53 \pm 4.27	n.s.
		(n=7)	(n=9)	
(65mM K ⁺ Kreb's) 2		70.55 \pm 4.94	80.13 \pm 5.93	n.s.
		(n=8)	(n=8)	
<u>Plateau pressure (mmHg)</u>				
('Normal' Kreb's) 1		31.26 \pm 1.41	29.67 \pm 1.03	n.s.
		(n=8)	(n=9)	
(65mM K ⁺ Kreb's) 2		41.54 \pm 1.97	42.30 \pm 2.58	n.s.
		(n=8)	(n=9)	
(65mM K ⁺ /3mM Ca ²⁺) 3		53.61 \pm 2.39	57.79 \pm 4.53	n.s.
		(n=8)	(n=9)	
<u>Time to plateau</u>				
<u>(minutes)</u>				
	1	13.46 \pm 1.19	13.34 \pm 1.57	n.s.
	2	10.82 \pm 1.28	8.89 \pm 1.25	n.s.
	3	21.54 \pm 1.98	21.93 \pm 3.50	n.s.

* Significance calculated using Student's t-test

Table 3.1(xiv)

Responses of the isolated perfused mesenteric vasculature from lean and dietary obese rats, after 8 weeks, to changes in the concentrations of potassium and calcium in the perfusing Kreb's solution (see Fig.14 for specimen trace). See section 2.2.1 for details of the different Kreb's solutions used. All values shown as mean \pm s.e.m.

	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
<u>Peak pressure (mmHg)</u>			
('Normal' Kreb's) 1	63.98 \pm 3.84 (n=8)	62.28 \pm 5.72 (n=6)	n.s.
(65mM K ⁺ Kreb's) 2	63.49 \pm 3.39 (n=8)	94.87 \pm 7.69 (n=10)	<0.001
<u>Plateau pressure (mmHg)</u>			
('Normal' Kreb's) 1	30.59 \pm 1.10 (n=8)	27.11 \pm 0.92 (n=10)	<0.001
(65mM K ⁺ Kreb's) 2	42.60 \pm 1.47 (n=8)	55.00 \pm 4.47 (n=10)	<0.001
(65mMK ⁺ /3mMCa ²⁺) 3	55.92 \pm 2.91 (n=8)	71.32 \pm 6.98 (n=10)	<0.005
<u>Time to plateau</u>			
<u>(minutes)</u>			
1	12.46 \pm 0.89	17.04 \pm 0.88	<0.001
2	10.25 \pm 1.09	16.27 \pm 1.53	<0.001
3	27.00 \pm 0.95	21.82 \pm 1.92	<0.001

* Significance calculated using Student's t-test

3.1.8 Observation of the effects in the rat of long term consumption of a varied palatable diet.

The weights and also details of food and water intake of rats given laboratory chow with or without a varied palatable diet for up to 16 weeks have been reported in sections 3.1.2 and 3.1.1, respectively. In this study changes in body weight and in food and water intake were monitored from 16 to 60 weeks in one group of 6 rats offered the palatable diet in addition to laboratory chow. The purposes of this study were two-fold. Firstly, to gain an indication of the degree of obesity which may be induced in this animal model and secondly, to study any long term trends in particular food preferences.

The weights (g) of the rats were monitored at various times throughout the study: 398.67 ± 25.62 after 16 weeks; 453.50 ± 32.22 after 25 weeks; 615.80 ± 44.53 after 42 weeks; 633.00 ± 45.18 after 48 weeks; 661.80 ± 45.00 after 52 weeks; 697.20 ± 38.79 after 59 weeks and 706.00 ± 39.87 after 60 weeks. Typical weights of lean and dietary obese rats up to 20 weeks are shown in Table 3.1(iii) and Fig.7. One of the rats of the group developed middle ear disease during week 30 and had to be killed. All readings after that period of time are therefore for 5 rats only.

The intake of the palatable food items altered during the course of the study. Table 3.1(xv) shows the 24-hour intake of the various food items (including chow) from weeks 16 to 60. Typical intake of dietary obese rats after 4, 8, and 12 weeks, taken from an earlier study are also included for comparison. It can be seen that the intake of chocolate increased considerably as the study progressed, with the 24-hour intake after 52 weeks being almost 10 times that

after 4 weeks. Intake of luncheon meat also generally increased with time, the intake after 60 weeks being approximately twice that after 4 weeks; although, there was a period between 12 and 16 weeks when very little luncheon meat was eaten. The intake of Rice Krispies fell slightly with time, being greatest after 4 weeks. The intake of the laboratory chow showed no particular trend throughout the study.

The daily intake of water (ml) showed no particular trend during the study. All the values are expressed as mean \pm s.e.m. for 7 days each week (the value per rat is shown, calculated from the volume consumed daily per group): after 16 weeks 23.80 ± 0.92 ; after 25 weeks 22.13 ± 0.86 ; after 48 weeks 23.57 ± 0.90 ; after 54 weeks 22.43 ± 0.37 ; after 56 weeks 22.71 ± 0.57 ; after 59 weeks 22.00 ± 0.62 and after 60 weeks 22.74 ± 1.19 . For water intake of lean and dietary obese rats up to 16 weeks see Table 3.1(11).

3.1.9 Weight, blood pressure and heart rate of naive (stock) Wistar rats of different ages (from 28 to 83 days after weaning).

To ascertain whether the effects of the handling and monitoring procedures altered the changes in weight, systolic blood pressure and heart rate which would normally occur with time in rats, naive Wistar rats were taken at random and single 'one off' sets of measurements of heart rate and blood pressure made in the usual way (see section 2.8.3). The rats were then weighed and returned to stock. The values obtained with these naive rats were then compared with those of age-matched lean trained rats, who had had their heart rates and blood pressures measured on several previous occasions. All rats were maintained on standard laboratory chow and water. The weights increased steadily with time (see Fig.16): 155.5 ± 7.2 at 28 days

after weaning; 210.5 ± 2.0 at 52 days; 223.6 ± 4.8 at 63 days; 237.7 ± 3.4 at 70 days and 252.5 ± 5.4 at 83 days. The value at 83 days after weaning was not significantly different from the value for the age-matched, trained, experimental animals (after 6 weeks chow) (254.3 ± 5.0), see Table 3.1(iii). Thus, the regular handling and monitoring procedures which the experimental animals were subjected to did not affect weight gain.

Blood pressure (mmHg) showed a slight tendency to increase with time (see Fig.15): 108.0 ± 2.9 at 28 days after weaning; 104.2 ± 1.7 at 52 days; 115.7 ± 3.4 at 63 days; 114.8 ± 4.7 at 70 days and 116.0 ± 3.0 at 83 days. The value at 70 days was similar to that of the age-matched, trained, experimental animals (after 4 weeks chow), which was 114.3 ± 3.3 mmHg. However, blood pressures of the experimental animals then increased rapidly and the value for the experimental animals after 6 weeks (chow-fed) was considerably greater than that of the age-matched naive stock rats (83 days after weaning); 137.9 ± 3.5 mmHg and 116.0 ± 3.0 mmHg, respectively (see also Table 3.1(iv)). Thus, it appears that the repeated monitoring of blood pressure and general handling may have induced some elevation in blood pressure. The heart rate (beats per minute) of the naive rats showed no particular trend with time/age: 358.4 ± 9.4 at 28 days after weaning; 350.2 ± 11.2 at 52 days; 341.2 ± 6.9 at 63 days; 326.3 ± 8.7 at 70 days and 358.6 ± 11.9 at 83 days. As with blood pressure values, the heart rates of the naive rats were considerably lower than those of the age-matched lean experimental (trained) rats (see Table 3.1(vii)). Thus, the handling and monitoring procedures may have induced some elevation in heart rate.

Table 3.1(xv)

Daily intake of the different food items by dietary obese rats throughout a 60 week study (g per rat).

	<u>Chow</u>	<u>Rice</u>	<u>Digestive</u>	<u>Luncheon</u>	<u>Chocolate</u>
<u>Week</u>		<u>Krispies</u>	<u>Biscuits</u>	<u>Meat</u>	
4	3.42	13.00	3.33	1.08	0.75
8	2.00	11.33	4.17	3.25	1.63
12	2.31	9.39	0.50	3.11	2.22
16	6.17	7.50	1.67	4.17	2.33
25	3.83	10.00	4.17	4.67	2.83
42	4.40	11.60	5.60	5.20	4.80
48	3.50	10.00	3.70	4.60	5.10
52	1.70	12.00	5.80	5.00	7.20
60	4.20	9.20	6.20	7.80	5.20

Fig. 15 Minimum systolic blood pressure (mmHg) of stock female Wistar rats of different ages (days since weaning).

All values shown as mean \pm s.e.m.
For "n" values see text, section 3.

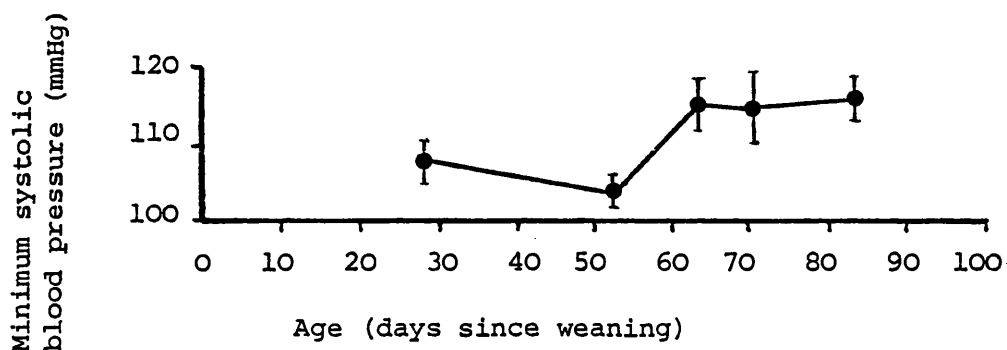
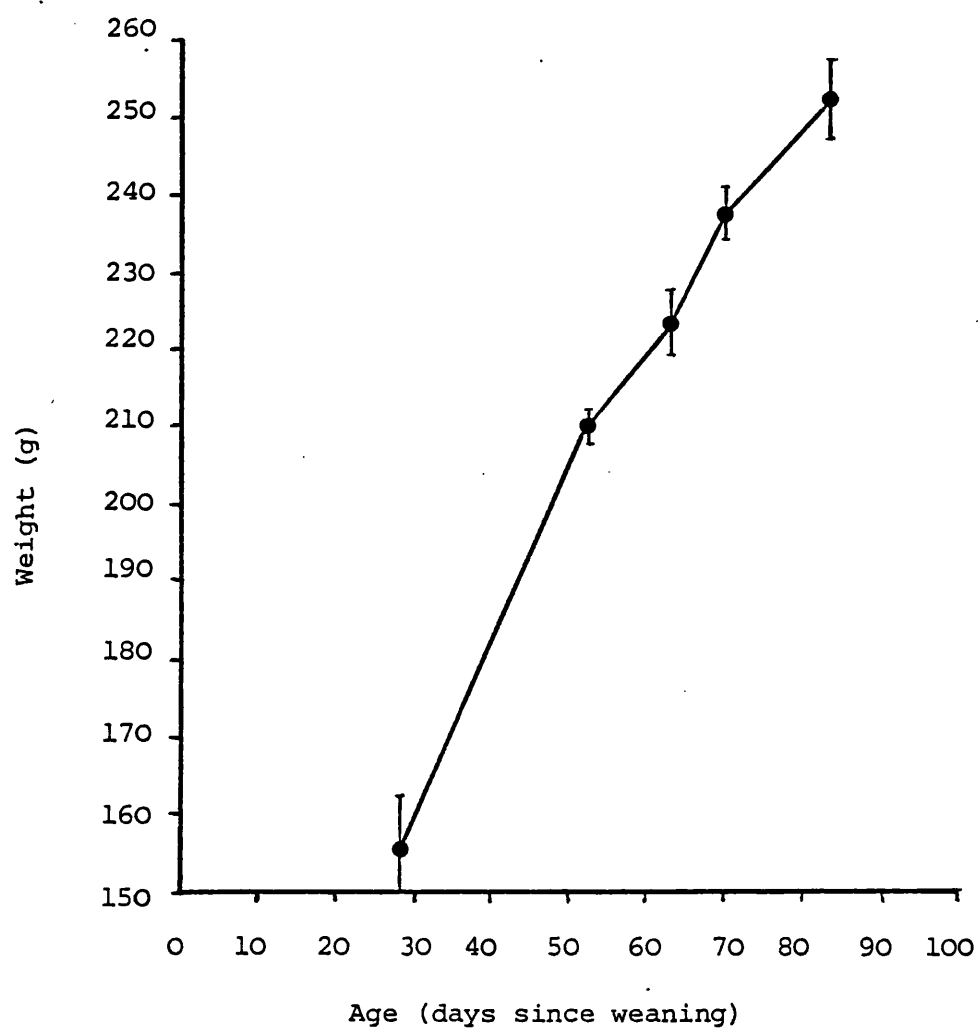


Fig. 16 Weights (g) of stock female Wistar rats of different ages (days since weaning).

All values shown as mean \pm s.e.m.
For "n" values see text, section 3.



3.2 THE EFFECTS OF DIFFERENT METHODS OF WEIGHT REDUCTION ON

CARDIOVASCULAR PARAMETERS

As stated in section 2.8.6, all groups except for the lean controls (L) received the palatable diet in addition to laboratory chow for the first 4 weeks of the study. For the second 4 week period the dietary obese group (D), the control-dosed obese group (CD), the amphetamine-treated group (AMPH), the fenfluramine-treated group (FEN) and the mazindol-treated group (MAZ) continued to receive the palatable diet, but group WD had the palatable diet withdrawn for the second 4 weeks of the study and received only chow. The lean controls continued to receive chow only throughout the second 4 weeks of the study.

3.2.1 Food and water intake following the withdrawal of the palatable diet or the administration of amphetamine, fenfluramine or mazindol.

The results for food and water intake in lean and dietary obese rats were discussed earlier (section 3.1.1).

a) Withdrawal of the palatable diet

Daily nutrient intake of rats who had had their palatable diet withdrawn (WD) was calculated and compared with values for chow-fed, age-matched lean controls (L) and dietary obese rats still receiving the palatable diet (D). The results are shown in Table 3.2(1). It can be seen from this table that the intake of all nutrients by group WD was approximately 50% of that of L during the first week following the withdrawal of the palatable diet. The food intake of group WD subsequently increased with time and by week 8 of this study approached that of the lean age-matched controls (L). Water intake of

group WD fell below that of the obese age-matched group (D) when the palatable diet was withdrawn (week 5), see Table 3.2(11), and remained constant throughout the remainder of the withdrawal period.

b) The effects of the anorectic drugs on food and water intake.

Food intake was not monitored regularly during the studies using the anorectic drugs, as their efficacy in reducing food intake has been well established. However, food intake was monitored for one 24-hour period for each drug-treated group (AMPH, FEN, MAZ) during the third week of drug administration. The anorectic effect of all three drugs can be clearly seen from Table 3.2(111). Amphetamine-treated and mazindol-treated groups showed a similar intake of most nutrients. However, food intake by the fenfluramine-treated rats was considerably greater (see section 4.5 for discussion of nutrient intake by AMPH, FEN, MAZ and WD).

Water intake was monitored during the second week of drug administration and the results were as follows: AMPH 16.49 ml per rat per day; FEN 15.48 ml per rat per day; MAZ 15.97 ml per rat per day, compared with 25.29ml per rat per day for dietary obese untreated rats and 18.44ml per rat per day for lean controls. Thus water intake was reduced in all drug-treated rats compared to age-matched lean and obese untreated rats.

Table 3.2(1)

Daily nutrient intake per rat during weeks 5 to 8 of study . WD= palatable diet plus chow for weeks 1-4, then palatable diet withdrawn for weeks 5-8; L= chow only for weeks 1-8; D= palatable diet plus chow for weeks 1-8.

	<u>WD</u>	<u>L</u>	<u>D</u>
<u>Week 5</u>			
Energy (kcal)	20.94	46.14	82.53
Protein (g)	1.33	2.99	1.94
Fat (g)	0.18	0.39	1.57
Carbohydrate (g)	4.18	9.42	17.33
Fibre (g)	0.26	0.60	0.96
Sodium (mg)	23.94	42.37	181.12
Potassium (mg)	55.00	109.80	57.42
Calcium (mg)	58.67	120.22	35.01
<u>Week 6</u>			
Energy (kcal)	24.75	41.14	80.54
Protein (g)	1.57	2.72	1.92
Fat (g)	0.21	0.33	1.55
Carbohydrate (g)	4.94	9.02	16.51
Fibre (g)	0.31	0.54	0.93
Sodium (mg)	24.03	44.02	169.98
Potassium (mg)	65.00	100.01	55.41
Calcium (mg)	69.33	112.37	32.68

CONTINUED OVERLEAF

Table 3.2(1) continued:

	<u>WD</u>	<u>L</u>	<u>D</u>
<u>Week 7</u>			
Energy (kcal)	29.98	42.31	80.69
Protein (g)	1.90	2.81	1.93
Fat (g)	0.25	0.34	1.57
Carbohydrate (g)	5.98	9.33	16.88
Fibre (g)	0.38	0.57	0.97
Sodium (mg)	34.63	43.44	173.52
Potassium (mg)	78.75	108.99	56.13
Calcium (mg)	84.00	116.41	33.04
<u>Week 8</u>			
Energy (kcal)	35.45	43.42	86.25
Protein (g)	2.25	2.75	1.94
Fat (g)	0.30	0.37	2.48
Carbohydrate (g)	7.07	8.66	15.11
Fibre (g)	0.45	0.55	0.93
Sodium (mg)	34.56	42.58	185.78
Potassium (mg)	93.13	114.06	51.18
Calcium (mg)	99.33	121.67	25.44

Table 3.2(ii)

Daily water intake (ml) per rat during weeks 5 to 8 of study.

	<u>WD</u>	<u>L</u>	<u>D</u>
Week 5	20.06	18.81	24.40
Week 6	19.46	19.53	26.59
Week 7	20.00	18.44	25.29
Week 8	21.08	18.11	24.60

Table 3.2(iii)

Daily intake of nutrients (per rat) during the third week of administration of the anorectic drug (week 7 of study). AMPH= amphetamine 5mg/kg daily; FEN= fenfluramine 10mg/kg daily; MAZ= mazindol 4mg/kg daily; D= dietary obese, untreated.

	<u>AMPH</u>	<u>FEN</u>	<u>MAZ</u>	<u>D</u>
Energy (kcal)	34.87	60.62	36.55	80.69
Protein (g)	0.93	1.48	0.91	1.93
Fat (g)	1.51	1.96	1.30	1.57
Carbohydrate (g)	4.83	10.04	5.75	16.88
Fibre (g)	0.26	0.63	0.34	0.97
Sodium (mg)	42.41	113.23	71.04	173.52
Potassium (mg)	31.39	41.56	28.19	56.13
Calcium (mg)	24.08	26.24	18.18	33.04

3.2.2 The effects of the various weight-reducing regimens on body weight

The weights of the rats are shown in Table 3.2(iv). There were small but significant differences in body weight between some of the groups at the start of week 1, therefore the changes in body weight during the study were used for the purpose of analysis (see Table 3.2(v) and Fig.17)a & b) for values). All groups receiving the palatable diet gained significantly more weight during the first 4 weeks of the study than the lean controls ($p < 0.01$ or less), though there was considerable variation in the actual weight gained by these groups with values ranging from $77.00 \pm 3.96\text{g}$ for group D to $48.67 \pm 4.79\text{g}$ for MAZ. During the second 4 weeks of the study the dietary obese group continued to gain more weight than the lean controls ($36.50 \pm 4.19\text{g}$ and $21.17 \pm 1.25\text{g}$), $p < 0.001$. The control orally-dosed obese group (CD) also gained more weight than the lean controls (25.87 ± 3.97), though this difference did not reach significance. All the rats in group WD lost weight during the second half of the study, the mean weight loss for the group was $19.00 \pm 2.94\text{g}$. While the mean weight loss for the group receiving amphetamine (AMPH) was similar to that of WD ($22.92 \pm 5.10\text{g}$), the weight changes within the group ranged from +10 to -54g, illustrating the considerable inter-individual variation in the response to amphetamine. All rats in the group which received fenfluramine (FEN) lost weight (mean weight loss $11.67 \pm 0.98\text{g}$). The weight change which occurred with mazindol administration was extremely variable, ranging from +9 to -36g, with the mean effect being a loss of $11.50 \pm 6.14\text{g}$.

Table 3.2(iv)

Weight (g) of rats at the start of week 1, and after 4 and 8 weeks of study. All values expressed as mean \pm s.e.m. for group. For abbreviations of groups see section 3.2

	<u>Start week 1</u>	<u>After 4 weeks</u>	<u>After 8 weeks</u>
L (n=12)	215.83 \pm 1.65	252.08 \pm 3.48	273.25 \pm 2.86
D (n=12)	214.58 \pm 2.30	291.58 \pm 4.99	328.08 \pm 8.52
CD (n=8)	207.12 \pm 3.22	277.25 \pm 5.92	303.13 \pm 7.84
WD (n=8)	206.25 \pm 3.52	263.88 \pm 3.00	244.88 \pm 2.32
AMPH (n=12)	208.33 \pm 2.05	269.58 \pm 7.23	246.67 \pm 7.04
FEN (n=12)	212.58 \pm 1.73	267.92 \pm 3.93	256.25 \pm 3.90
MAZ (n=6)	212.00 \pm 1.79	262.33 \pm 6.43	251.17 \pm 7.56

Table 3.2(v)

Changes in weight (g) of groups of rats during the first and second four week periods. All values expressed as mean \pm s.e.m. for group.

	<u>Start Week 1</u> <u>to End Week 4</u>	<u>Start Week 5</u> <u>to End Week 8</u>
L (n=12)	+36.25 \pm 2.99	+21.17 \pm 1.25
D (n=12)	+77.00 \pm 3.96 **	+36.50 \pm 4.19 **
CD (n=8)	+70.12 \pm 5.49 **	+25.87 \pm 3.97
WD (n=8)	+57.62 \pm 3.96 **	-19.00 \pm 2.94
AMPH (n=12)	+61.25 \pm 5.73 **	-22.92 \pm 5.10
FEN (n=12)	+55.33 \pm 4.15 **	-11.67 \pm 0.98
MAZ (n=6)	+48.67 \pm 4.79 *	-11.50 \pm 6.14

* significantly greater than L $p < 0.01$

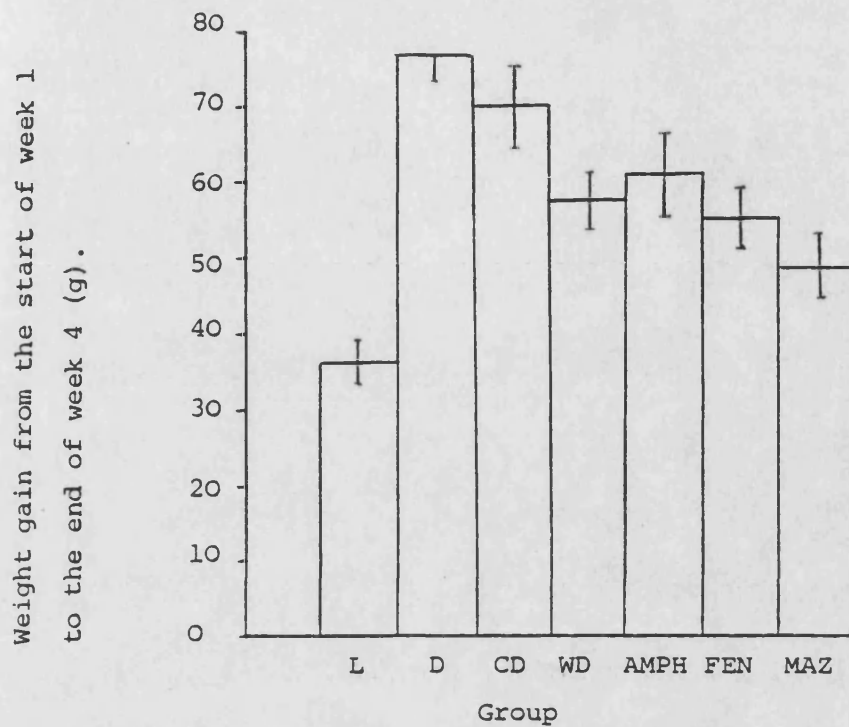
** significantly greater than L $p < 0.001$

(Significance calculated using Dunnett's test)

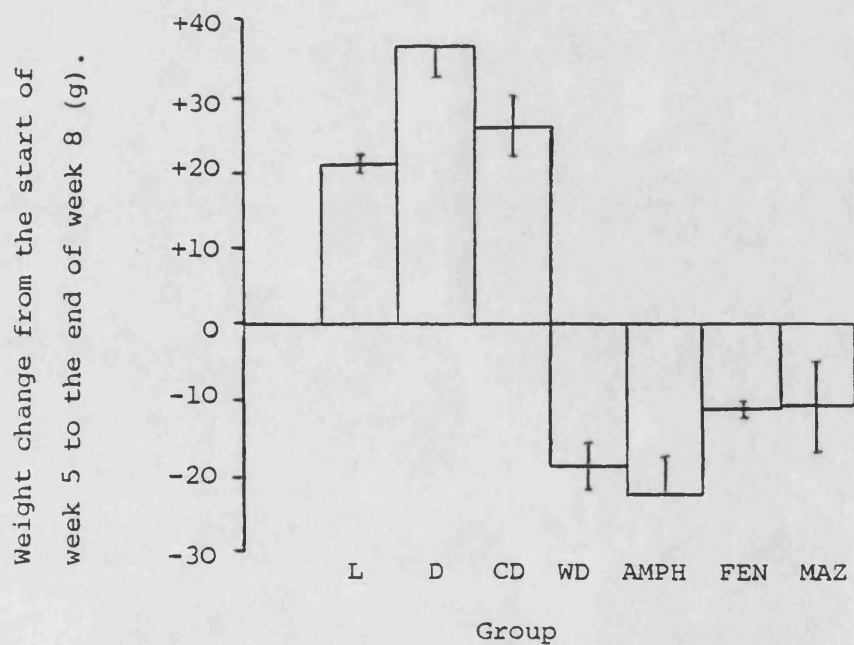
Fig. 17

a) Body weight gain during the first 4 weeks of the study (g).

All values expressed as mean \pm s.e.m.



b) The effect of withdrawal of the palatable diet or treatment with anorectic drugs upon body weight change during the second 4 weeks of the study. All values mean \pm s.e.m.



3.2.3 The effects of the different methods of weight reduction on minimum systolic blood pressure.

The minimum systolic blood pressure values are shown in Table 3.2(vi) and illustrated in Fig.18. From these data it can be seen that the blood pressures of the groups were dissimilar at the start of week 1, therefore the changes in blood pressure occurring during the different experimental periods were used for analysis (see Table 3.2(vii)). Minimum systolic blood pressure increased for all groups during the first 4 weeks of the study, the rate of increase being greater in those groups which received the palatable diet (D, CD, WD, AMPH, FEN and MAZ) than in the chow-fed group (L) (see Table 3.2(vii) and Fig.18).

During the second 4 week period groups D and CD maintained their elevated blood pressures (141.00 ± 4.47 and 142.62 ± 3.54 mmHg, respectively). The mean change in blood pressure for D during this period was -0.67 ± 4.16 mmHg and for CD, $+2.62 \pm 4.18$ mmHg. The mean blood pressure for the lean group remained stable over the second 4 week period, with a mean change of $+1.17 \pm 3.89$ mmHg. The blood pressure of group WD fell by 14.37 ± 7.37 mmHg during the 4 week period of withdrawal of the palatable diet, bringing it to the same level as that of group L. The range of change in blood pressure for group WD was -43 to +15mmHg (two rats showed increases of 10.5 and 15 mmHg respectively). Considerable variation was seen between the changes in systolic blood pressure of the three groups receiving the anorectic drugs. AMPH showed only a modest fall in blood pressure (-4.55 ± 4.37 mmHg). The use of mean values obscures the fact that increases in blood pressure (13.5, 14 and 2 mmHg) were seen in three rats of this group. FEN showed a significantly greater reduction in

blood pressure than any of the other groups ($p < 0.001$) with a mean fall of 27.32 ± 3.68 mmHg. All the rats in the group showed a fall in blood pressure of at least 10mmHg and in three rats the fall was 40mmHg or greater. MAZ showed a small increase in blood pressure for the group as a whole ($+4.50 \pm 8.93$ mmHg). As for AMPH, the use of the mean value obscures the fact that considerable increases in blood pressure were seen in half of this small group of rats (12, 24 and 32 mmHg) whilst the other half of the group showed reductions (5, 11 and 25 mmHg).

The correlation coefficient (r) was calculated for changes in weight and changes in blood pressure for all groups during the different stages of the study (see Table 3.2(viii)). Only in group CD was a significant positive correlation seen between changes in weight and blood pressure during the first 4 weeks of the study. However, during the second half of the study, a strong negative correlation was seen between changes in blood pressure and changes in weight in groups AMPH and MAZ. The rats which lost the most weight in these groups showed the greatest elevation in blood pressure (see section 4.5 for further discussion).

3.2.4 The effects of the different methods of weight reduction on 'in vivo' heart rate.

The heart rates of the various groups were dissimilar at the start of week 1 (see Table 3.2(ix)), therefore the absolute values are useful only to observe changes occurring within a group and general trends. The changes in heart rate occurring during the different stages of the experiment were therefore used for comparisons between groups (see Table 3.2(x)). During the first 4

weeks of the study all the groups of rats receiving the palatable diet showed greater increases in heart rate than lean controls, though this difference only reached significance for groups MAZ and CD ($p < 0.001$) and D ($p < 0.01$), the first two of these groups showing large increases in heart rate (47.25 ± 5.28 and 48.46 ± 8.68 beats per minute, respectively).

During the second 4 week period of the study the dietary obese group (D) again showed a greater increase in heart rate than lean controls (17.29 ± 7.92 and 7.37 ± 7.08 beats per minute), though this difference was not significant. Group CD (control-dosed obese) showed a small fall in heart rate (-11.37 ± 8.33 beats per minute). The weight-reduced groups WD, AMPH and FEN all showed significantly greater reductions in heart rate (beats per minute) than dietary obese (D) and lean (L) groups : WD (-19.50 ± 8.54); AMPH (-44.30 ± 10.65) and FEN (-36.32 ± 6.45). The mazindol-treated group, however, showed a large increase in heart rate (74.30 ± 13.10 beats per minute) with 5 out of the 6 rats in the group having a heart rate of greater than 420 beats per minute and one rat having a heart rate of 505 beats per minute. Interestingly, this rat also showed the greatest weight loss of the group, and there was a significant negative correlation ($p < 0.05$) for the group as a whole between change in heart rate and change in body weight during the second 4 weeks of the study (see Table 3.2(xi)). Thus these data show that mazindol can cause tachycardia. The correlation coefficient (r) was calculated for all groups, between weight change and change in heart rate during the different periods of the experiment. The values are shown in Table 3.2(xi). However, the only significant correlation found was for the mazindol-treated rats during the second half of the study.

Table 3.2(vi)

Minimum systolic blood pressures (mmHg) of rats at start of week 1 and after 4 and 8 weeks of study. All values expressed as mean \pm s.e.m. for group. For abbreviations used see section 3.2.

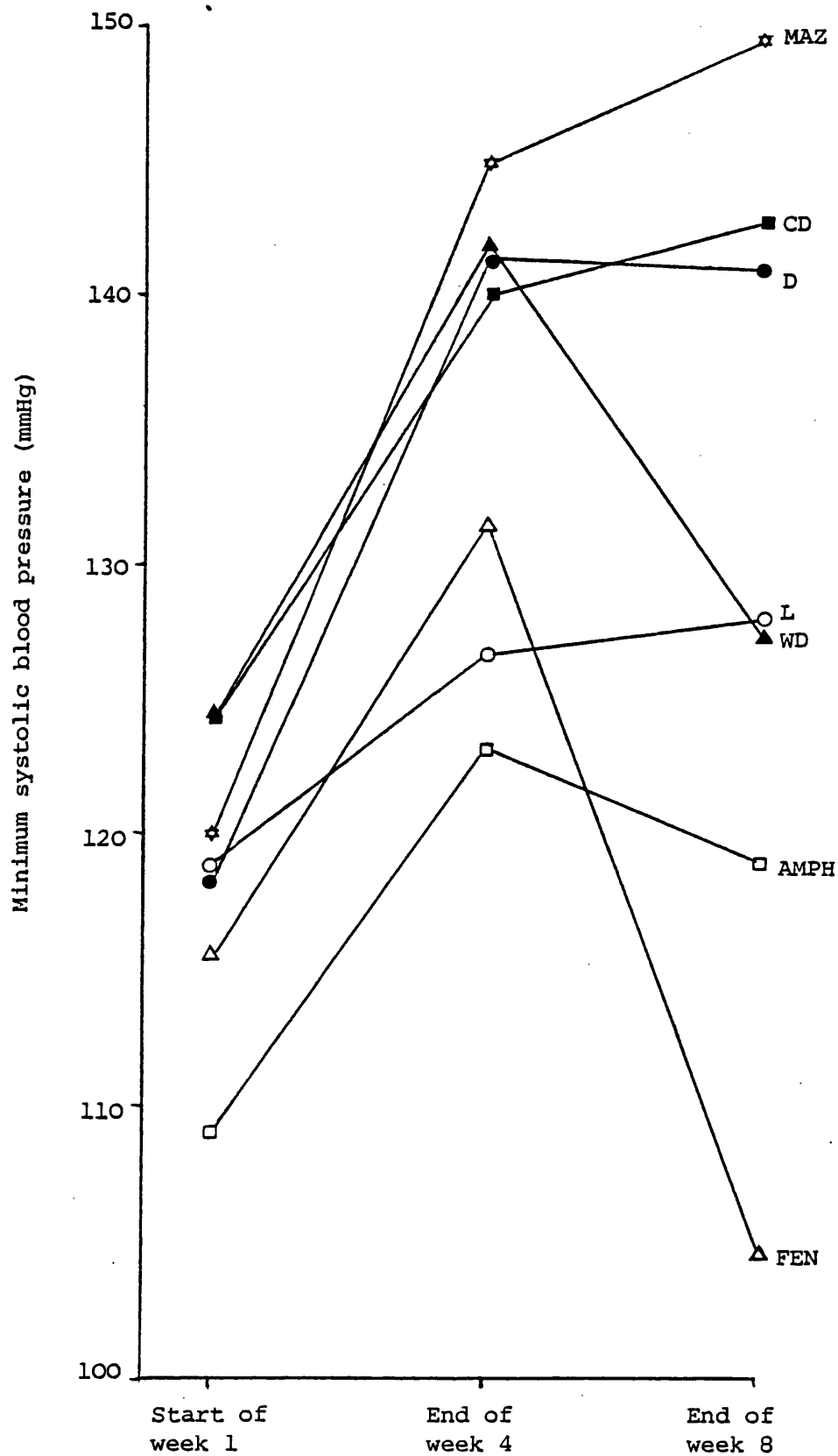
	<u>Start Week 1</u>	<u>After 4 weeks</u>	<u>After 8 weeks</u>
L (n=12)	118.83 \pm 2.32	126.83 \pm 3.04	128.00 \pm 2.57
D (n=12)	118.33 \pm 3.81	141.67 \pm 3.29	141.00 \pm 4.47
CD (n=8)	124.37 \pm 3.16	140.00 \pm 4.45	142.62 \pm 3.54
WD (n=8)	124.37 \pm 4.98	141.87 \pm 5.31	127.50 \pm 3.26
AMPH (n=12)	109.04 \pm 2.21	123.25 \pm 2.62	118.91 \pm 4.79
FEN (n=12)	115.46 \pm 3.03	131.77 \pm 3.34	104.45 \pm 3.30
MAZ (n=6)	120.00 \pm 1.78	144.83 \pm 5.41	149.33 \pm 7.33

Table 3.2(vii)

Changes in minimum systolic blood pressures (mmHg) of rats during the first and second four week periods. All values expressed as mean \pm s.e.m. for group.

	<u>Start Week 1</u> <u>to End Week 4</u>	<u>Start Week 5</u> <u>to End Week 8</u>
L (n=12)	+8.00 \pm 3.04	+1.17 \pm 3.89
D (n=12)	+23.33 \pm 3.86	-0.67 \pm 4.16
CD (n=8)	+15.62 \pm 5.16	+2.62 \pm 4.18
WD (n=8)	+17.50 \pm 4.64	-14.37 \pm 7.37
AMPH (n=12)	+14.21 \pm 2.69	-4.55 \pm 4.37
FEN (n=12)	+15.09 \pm 3.89	-27.32 \pm 3.68
MAZ (n=6)	+24.83 \pm 5.70	+4.50 \pm 8.93

Fig. 18 The effect of different methods of weight reduction on minimum systolic blood pressure.



All values shown as mean for group. Error bars omitted, see Table 3.2(vi) for s.e.m. values.

Table 3.2(viii)

Correlation coefficient (r) for changes in weight (g) and changes in blood pressure (mmHg) of rats during the first and second four week periods of the study. For abbreviations see section 3.2

	<u>Start Week 1</u> <u>to End Week 4</u>	<u>Start Week 5</u> <u>to End Week 8</u>
L (n=12)	-0.1703	-0.0693
D (n=12)	-0.3081	0.0476
CD (n=8)	0.7557 *	-0.4204
WD (n=8)	-0.1044	-0.0609
AMPH (n=12)	0.1977	-0.7898 **
FEN (n=12)	-0.1742	-0.3365
MAZ (n=6)	0.5162	-0.9324 **

* significant $p < 0.05$

** significant $p < 0.01$

Table 3.2(1x)

'In vivo' heart rate (beats per minute) of rats at start of week 1 and after 4 and 8 weeks. All values expressed as mean \pm s.e.m. for group. For abbreviations see section 3.2.

	<u>Start Week 1</u>	<u>After 4 weeks</u>	<u>After 8 weeks</u>
L (n=12)	377.33 \pm 10.24	379.33 \pm 8.14	386.71 \pm 10.59
D (n=12)	379.04 \pm 6.21	399.04 \pm 7.22	415.50 \pm 7.52
CD (n=8)	354.14 \pm 8.22	402.62 \pm 7.24	398.12 \pm 8.67
WD (n=8)	364.69 \pm 7.44	383.90 \pm 10.17	364.44 \pm 10.70
AMPH (n=12)	360.21 \pm 5.04	375.18 \pm 8.25	336.14 \pm 5.43
FEN (n=12)	350.62 \pm 8.35	368.64 \pm 7.00	332.32 \pm 7.91
MAZ (n=6)	326.50 \pm 5.80	373.75 \pm 10.05	448.05 \pm 16.20

Table 3.2(x)

Changes in heart rate (beats per minute) during first and second weeks of the experiment. All values expressed as mean \pm s.e.m. for group.

	<u>Start Week 1</u> <u>to End Week 4</u>	<u>Start Week 5</u> <u>to End Week 8</u>
L (n=12)	+2.00 \pm 9.33	+7.37 \pm 7.08
D (n=12)	+20.00 \pm 7.25	+17.29 \pm 7.92
CD (n=8)	+48.46 \pm 8.68	-11.37 \pm 8.33
WD (n=8)	+19.25 \pm 11.00	-19.50 \pm 8.54
AMPH (n=12)	+14.97 \pm 8.76	-44.30 \pm 10.65
FEN (n=12)	+13.86 \pm 6.93	-36.32 \pm 6.45
MAZ (n=6)	+47.25 \pm 5.28	+74.30 \pm 13.10

Table 3.2(xi)

Correlation coefficient (r) for changes in weight (g) and changes in heart rate (beats per minute) of rats during the first and second four week periods of study. For abbreviations see section 3.2

	<u>Start Week 1</u>	<u>Start Week 5</u>
	<u>to End Week 4</u>	<u>to End Week 8</u>
L (n=12)	0.4582	0.0186
D (n=12)	-0.3807	-0.5137
CD (n=8)	-0.2437	0.3096
WD (n=8)	0.0056	-0.1856
AMPH (n=12)	0.2003	0.3691
FEN (n=12)	0.5258	0.4288
MAZ (n=6)	-0.5594	-0.8131 *

* significant $p < 0.05$

3.2.5 'In vitro' studies: the isolated perfused heart preparation

After the 8 weeks of the experiment the rats were killed and the isolated heart and mesenteric vasculature studied. The 'in vitro' data for the lean (L) and dietary obese (D) groups have already been presented in section 3.1 as results for lean and obese rats after 8 weeks.

a) The responses to doses of noradrenaline

The baseline beating rate was similar for hearts from L and D; while the beating rates for hearts from all the weight-reduced groups (WD, AMPH, FEN, MAZ) were lower than those for L and D (see Table 3.2(xii)). The differences only reached statistical significance for the weight-reduced groups compared with L ($p < 0.05$ or less) but not compared with D.

The response of the isolated perfused heart to noradrenaline was dose-dependent for all groups. In addition, responses of hearts from D tended to be greater than those of hearts from L, though this difference was not significant. The responses to the lowest dose of noradrenaline were similar for hearts from L, WD and AMPH, but were significantly greater for hearts from FEN and MAZ compared to L ($p < 0.01$ and $p < 0.05$ respectively). The responses of the hearts to the middle dose of noradrenaline were similar for all groups, although responses tended to be greater for hearts from D, FEN and MAZ than for L, WD and AMPH. There was no significant difference between responses of hearts from L and D at the highest dose level of noradrenaline, but the responses of hearts from all the weight-reduced groups tended to be smaller, the difference reaching statistical significance for WD, AMPH and FEN compared with D ($p < 0.01$) and for AMPH compared with L ($p < 0.05$).

Table 3.2(x11)

Response of beating rate (beats per minute) of isolated perfused rat heart to doses of noradrenaline. All values expressed as mean \pm s.e.m. for group. For abbreviations see section 3.2

	<u>Baseline</u>	<u>Response to dose of noradrenaline (increase</u>		
	<u>beating rate</u>	<u>in beating rate)</u>		
		<u>5×10^{-11} mole</u>	<u>5×10^{-10} mole</u>	<u>5×10^{-9} mole</u>
L	279.44 \pm 8.06	10.00 \pm 3.63	47.78 \pm 13.13	102.78 \pm 10.51
(n=9)				
D	275.45 \pm 13.31	19.09 \pm 5.59	63.18 \pm 12.55	113.18 \pm 13.40
(n=11)				
WD	256.67 \pm 9.32	13.89 \pm 4.23	47.22 \pm 9.43	78.89 \pm 11.78
(n=9)				
AMPH	250.56 \pm 6.99	15.56 \pm 6.21	46.11 \pm 7.54	75.56 \pm 6.79
(n=9)				
FEN	262.27 \pm 4.39	26.36 \pm 2.70	53.64 \pm 7.75	82.27 \pm 7.21
(n=11)				
MAZ	259.00 \pm 15.10	27.00 \pm 10.32	61.00 \pm 21.06	94.00 \pm 21.59
(n=5)				

Next, considering the contractility of the isolated perfused hearts (under 2g tension), see Table 3.2(xiii): baseline contractility (g) was greater for hearts from groups L and WD than for hearts from the other groups. This was significant for hearts from L (11.78 ± 1.02) compared with D (7.93 ± 0.64) $p < 0.001$; AMPH (9.50 ± 0.45) $p < 0.01$; FEN (7.64 ± 0.84) $p < 0.001$ and MAZ (8.05 ± 0.92) $p < 0.001$. The difference was also significant for hearts from WD (10.11 ± 1.17) compared with D ($p < 0.001$), FEN ($p < 0.001$) and MAZ ($p < 0.05$). Hearts from D had a similar baseline contractility to hearts from FEN and MAZ, though significantly smaller baseline contractility than WD ($p < 0.001$), L ($p < 0.001$) and AMPH ($p < 0.05$).

The response of contractility (as % baseline contractility) of the hearts to noradrenaline was dose-dependent for all groups except FEN and MAZ (see Table 3.2(xiii)). A dose-dependent relationship was not apparent for these two groups, as the response to noradrenaline of hearts from MAZ appeared to be maximal with the lowest dose, with no significant increase after that dose. The response of hearts from FEN appeared to reach maximum with the middle dose (5×10^{-10} mole) of noradrenaline with no significant increase after that dose. The maximal response for the hearts from all the other groups did not occur until the highest dose, thus there appeared to be an increased sensitivity to noradrenaline in hearts from MAZ and FEN (see section 4.5 for discussion). The response to noradrenaline was greater for hearts from dietary obese rats than for hearts from lean rats at all three doses, although the difference only reached significance with the highest dose ($p < 0.05$). The responses to noradrenaline were similar in hearts from L, WD and AMPH for all dose levels, while hearts from FEN and MAZ consistently gave the greatest responses to

all doses of noradrenaline, again showing evidence of a heightened response to noradrenaline in the hearts from these two groups.

The number of arrhythmias occurring after the three doses of noradrenaline are shown in Table 3.2(xiv). No significant differences were found between the groups.

b) The responses to doses of acetylcholine

The responses to acetylcholine (5×10^{-9} and 5×10^{-8} mole) were expressed as the duration of cardiac arrest and the recovery time (time to recover regular beat). Both of these parameters were dose-dependent for hearts from all groups (see Table 3.2(xv)).

The duration of cardiac arrest and recovery time tended to be longer for hearts from obese rats than for hearts from lean controls, with both doses of acetylcholine. However, only the differences in recovery time reached significance, $p < 0.01$. All responses of hearts from AMPH were similar to those from L. Responses of hearts from WD tended to be greater than those from L, although the differences did not reach significance. Responses of hearts from FEN were greater than those of hearts from L ($p < 0.05$ or less) for all responses except the duration of cardiac arrest with the higher dose.

Table 3.2(xiii)

Response of contractility of isolated perfused rat heart to doses of noradrenaline (under 2g basal tension). All values expressed as mean \pm s.e.m. for group. For abbreviations see section 3.2.

	<u>Baseline</u>	<u>Response to dose of noradrenaline (% baseline</u>		
	<u>contractility</u>	<u>contractility).</u>		
	<u>(g)</u>	<u>5×10^{-11} mole</u>	<u>5×10^{-10} mole</u>	<u>5×10^{-9} mole</u>
L	11.78 \pm 1.02	144.62 \pm 4.21	155.21 \pm 7.92	170.88 \pm 8.76
(n=9)				
D	7.93 \pm 0.64	151.60 \pm 6.17	176.09 \pm 9.68	195.63 \pm 9.99
(n=11)				
WD	10.11 \pm 1.17	140.82 \pm 4.97	176.11 \pm 14.29	192.01 \pm 14.44
(n=9)				
AMPH	9.50 \pm 0.45	150.06 \pm 5.92	165.88 \pm 8.85	175.18 \pm 8.49
(n=9)				
FEN	7.64 \pm 0.84	185.85 \pm 20.83	218.76 \pm 32.47	197.50 \pm 17.21
(n=11)				
MAZ	8.05 \pm 0.92	190.30 \pm 12.60	187.93 \pm 17.25	201.18 \pm 17.49
(n=5)				

Table 3.2(xiv)

The number of arrhythmias (ectopic beats and missed beats) occurring in the isolated perfused rat hearts after three doses of noradrenaline (values for each preparation shown). For abbreviations see section 3.2

	<u>Dose of noradrenaline</u>		
	<u>5×10^{-11} mole</u>	<u>5×10^{-10} mole</u>	<u>5×10^{-9} mole</u>
L (n=9)	0,8,0,0,0,0, 2,0,0	0,3,1,4,14,0, 6,6,0	10,0,0,63,6,0, 7,0,35
D (n=11)	0,0,0,0,0,0, 0,1,0,0,0	0,0,1,0,0,0, 2,10,1,0,0	0,0,1,1,0,0, 0,1,2,0,0
WD (n=9)	0,0,0,0,0,0, 0,1,2	0,0,2,0,0,85, 0,1,1	0,1,0,0,0,27, 0,0,16
AMPH (n=9)	0,0,10,0,0,0, 0,0,0	1,1,0,0,4,0, 0,0,1	0,1,1,4,0,0, 0,0,0
FEN (n=11)	0,1,0,0,0,0, 0,0,0,1,0	0,0,0,2,0,2, 0,0,0,0,0	4,0,51,0,0,6, 0,0,0,1,0
MAZ (n=5)	0,0,0,2,4	3,0,0,1,0	17,0,0,3,0

No significant differences were found between the groups for any dose of noradrenaline (using the Mann-Whitney U-test).

Table 3.2(xv)

Responses of isolated perfused hearts from groups of rats to two doses of acetylcholine. All values shown as mean \pm s.e.m. for group.

a) Duration of cardiac arrest (seconds)

<u>Group</u>	<u>Dose of acetylcholine</u>	
	<u>5×10^{-9} mole</u>	<u>5×10^{-8} mole</u>
L (n=9)	3.84 \pm 0.94	8.56 \pm 1.38
D (n=11)	5.54 \pm 0.90	10.87 \pm 1.19
WD (n=9)	4.64 \pm 0.84	13.82 \pm 1.95
AMPH (n=9)	3.54 \pm 0.82	10.11 \pm 1.31
FEN (n=11)	6.49 \pm 1.71	14.22 \pm 6.84
MAZ (n=5)	6.26 \pm 2.00	18.50 \pm 3.17

b) Length of time to recover regular beat (seconds)

<u>Group</u>	<u>Dose of acetylcholine</u>	
	<u>5×10^{-9} mole</u>	<u>5×10^{-8} mole</u>
L (n=9)	11.12 \pm 2.03	37.66 \pm 2.27
D (n=11)	16.62 \pm 1.29	46.15 \pm 4.14
WD (n=9)	14.06 \pm 2.67	41.89 \pm 3.26
AMPH (n=9)	11.72 \pm 1.84	39.68 \pm 2.18
FEN (n=11)	20.17 \pm 2.33	50.93 \pm 5.89
MAZ (n=5)	10.66 \pm 2.80	34.94 \pm 2.64

For key to abbreviations for groups see section 3.2

3.2.6 'In vitro' studies:the isolated perfused mesenteric vasculature

a) The responses to doses of noradrenaline

The maximum responses to noradrenaline, expressed as maximum increase in perfusion pressure (mmHg), of the isolated perfused mesenteric vasculature from lean, dietary obese and weight-reduced groups were determined. The maximum response was smaller for tissues from all weight-reduced groups than for tissues from the dietary obese group ($p < 0.01$ or less): D (171.70 ± 3.42); L (156.90 ± 4.28); WD (96.33 ± 7.69); AMPH (139.34 ± 11.74); FEN (143.29 ± 10.42); MAZ (82.37 ± 14.11). These results are shown graphically in Fig.19. The maximal responses of the mesenteric vasculature from WD and MAZ were also significantly smaller than for tissues from L ($p < 0.001$). There was no significant correlation between body weight (g) and maximal increase in perfusion pressure for any of the groups. The correlation coefficient (r) for each group is as follows: L (-0.2792); D (0.1800); WD (0.4761); AMPH (0.0314); FEN (0.0917); MAZ (-0.1062).

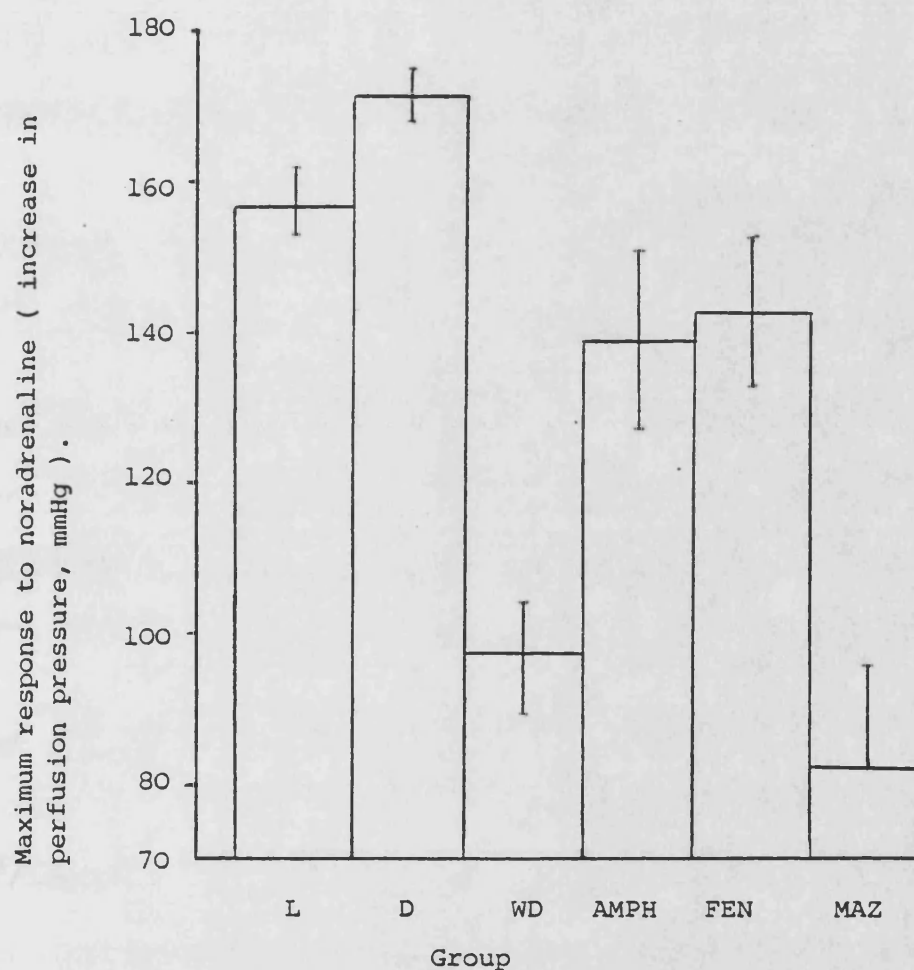
b) The responses to changes in the concentrations of potassium and calcium in the perfusing Kreb's solution.

See section 3.1.7d) and sample trace Fig.14 for definitions of parameters recorded. The effects of weight reduction by withdrawal of the palatable diet (WD) were investigated. The peak pressure obtained with 'normal' Kreb's solution (Peak Pressure 1) for tissues from WD was similar to that for tissues from age-matched lean and dietary obese animals (see Table 3.2(xvi)). The peak pressure obtained with 65mM K^+ Kreb's solution (Peak Pressure 2) for tissues from group WD were significantly lower than for tissues from obese rats ($p < 0.01$), the value falling between those of tissues from lean and obese animals. Plateau pressures 2 and 3 were lower for tissues from WD

than for tissues from the obese rats (both $p < 0.001$), and also lower than those from lean rats ($p < 0.05$) (although these particular differences were significant, they were small). The times to Plateaux 1 and 2 were similar for tissues from WD and D, however, there was a small but significant increase in the time to Plateau 3 for tissues from WD compared to D ($p < 0.05$). The time to Plateau 3 was similar for tissues from WD and L, although the times to Plateaux 1 and 2 were greater for tissues from WD than from L ($p < 0.001$ and $p < 0.01$, respectively).

Fig. 19 The effect of different methods of weight reduction on the maximum response of the isolated perfused mesenteric vasculature of the rat to noradrenaline. Response expressed as increase in perfusion pressure, mmHg.

All values shown as mean \pm s.e.m.



Key

L = Lean (8 weeks)

D = Dietary obese (8 weeks)

WD = Palatable diet withdrawn

AMPH = Amphetamine 5mg/kg daily

FEN = Fenfluramine 10mg/kg daily

MAZ = Mazindol 4mg/kg daily

Table 3.2(xvi)

Comparison of the responses of the isolated perfused mesenteric vasculature from previously obese rats, which then had the palatable diet withdrawn for 4 weeks (WD), with those from age-matched lean (L) and dietary obese rats (D) to changes in the concentrations of potassium and calcium in the perfusing Kreb's solution. See section 2.2.1 for details of the different Kreb's solutions used. All values expressed as mean \pm s.e.m.

	<u>WD</u>	<u>Lean</u>	<u>Obese</u>
<u>Peak pressure (mmHg)</u>			
('Normal' Kreb's) 1	66.92 \pm 8.84	63.98 \pm 3.84	62.28 \pm 5.72
	(n=7)	(n=8)	(n=6)
(65mM K ⁺ Kreb's) 2	72.63 \pm 5.08	63.49 \pm 3.39	94.87 \pm 7.67
	(n=10)	(n=8)	(n=10)
<u>Plateau pressure (mmHg)</u>			
('Normal' Kreb's) 1	28.29 \pm 1.15	30.59 \pm 1.10	27.11 \pm 0.92
	(n=10)	(n=8)	(n=10)
(65mM K ⁺ Kreb's) 2	38.69 \pm 1.47	42.60 \pm 1.47	55.00 \pm 4.47
	(n=10)	(n=8)	(n=10)
(65mMK ⁺ /3mM Ca ²⁺) 3	49.21 \pm 2.61	55.92 \pm 2.91	71.32 \pm 6.98
	(n=10)	(n=8)	(n=10)
<u>Time to plateau</u>			
<u>(minutes)</u>			
1	18.87 \pm 1.39	12.46 \pm 0.89	17.04 \pm 0.88
2	14.60 \pm 1.74	10.25 \pm 1.09	16.27 \pm 1.53
3	25.33 \pm 1.25	27.00 \pm 0.95	21.82 \pm 1.92

NB. Values for Lean and Obese group have been discussed earlier, see Table 3.1(xiv) and section 3.1.7 d) for details.

3.3 THE EFFECTS OF THE OESTROUS CYCLE UPON BODY WEIGHT AND MINIMUM SYSTOLIC BLOOD PRESSURE IN LEAN AND DIETARY OBESE RATS.

3.3.1 Changes in body weight during the oestrous cycle of the rat

Cyclical changes in weight (g) were observed in both lean and dietary obese rats during the oestrous cycle (see Fig.20). Body weights were compared relative to body weight during the oestrus phase of the cycle. In lean rats the body weight was at its lowest in the oestrus phase, with the values (g) for metoestrus ($+1.56 \pm 0.45$) for dioestrus ($+2.42 \pm 0.59$) and pro-oestrus ($+2.10 \pm 0.54$). However, for dietary obese rats the pattern was different. Body weights were lowest in the metoestrus phase (-2.83 ± 0.60), followed by dioestrus (-1.00 ± 0.59). The value for pro-oestrus was similar to that for oestrus ($+0.08 \pm 0.60$).

The maximum variation in body weight across the oestrous cycle was less than 3g for both lean and obese rats. This value was smaller than the average weekly weight gain for lean rats (approximately 5g) and considerably smaller than the average weight gain for the obese rats (often at least 10g), so this factor was not considered in the later studies. However, it may account for a small part of the variations in weight gain observed.

3.3.2 Changes in systolic blood pressure during the oestrous cycle of the rat.

Minimum systolic blood pressure readings have been used for calculation purposes throughout these studies (that is, the minimum value obtained for each rat during any one monitoring period). For lean rats the variation in minimum systolic blood pressure across the cycle was approximately 4mmHg (see Fig.21a). For the dietary obese

rats the variation was approximately 8mmHg (see Fig.21b).

The differences in minimum systolic blood pressure readings observed between groups of dietary obese rats were approximately 20mmHg (see Fig.8) and it was therefore considered unlikely that the variations seen throughout the oestrous cycle would have affected these results, though they may have been responsible for some of the variations seen.

The two groups of rats were not age-matched and the lean rats (approximately 17 weeks of age) were older than the dietary obese rats (approximately 14 weeks). Therefore, the actual values of minimum systolic blood pressure of the two groups can not be compared directly because systolic blood pressure rises with time for both lean and dietary obese Wistar rats (see Fig.8). There was no reason to suppose that the oestral rhythms of weight and blood pressure would be affected by this 3 week age difference, as all the rats were sexually mature throughout the experiment.

Fig.20 Body weight variations (with respect to weight during oestrus phase of cycle) during the different stages of the oestrous cycle in lean and dietary obese rats.

All values mean \pm s.e.m.

Key: OEST = oestrus ; MET = metoestrus ; DI = dioestrus ;

PRO = pro-oestrus

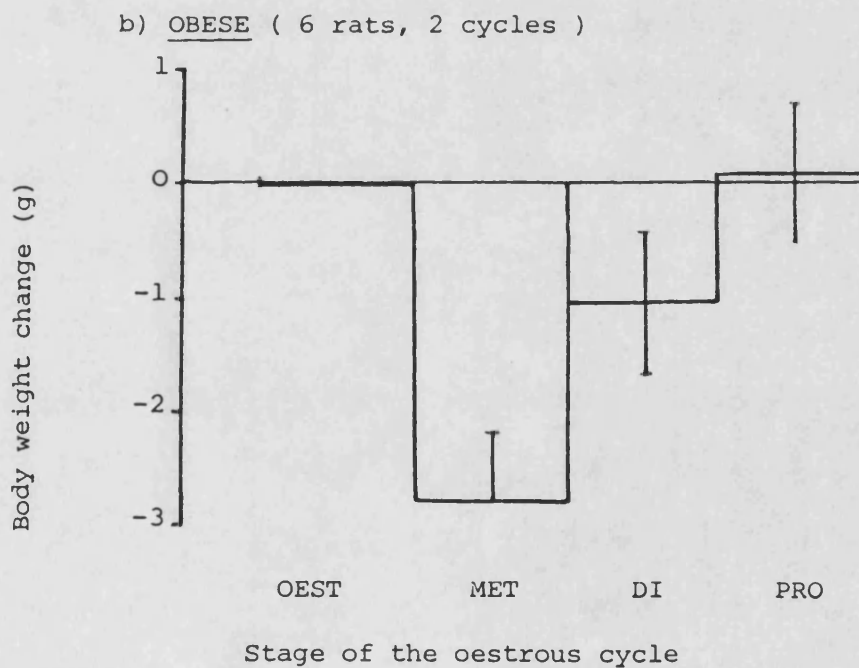
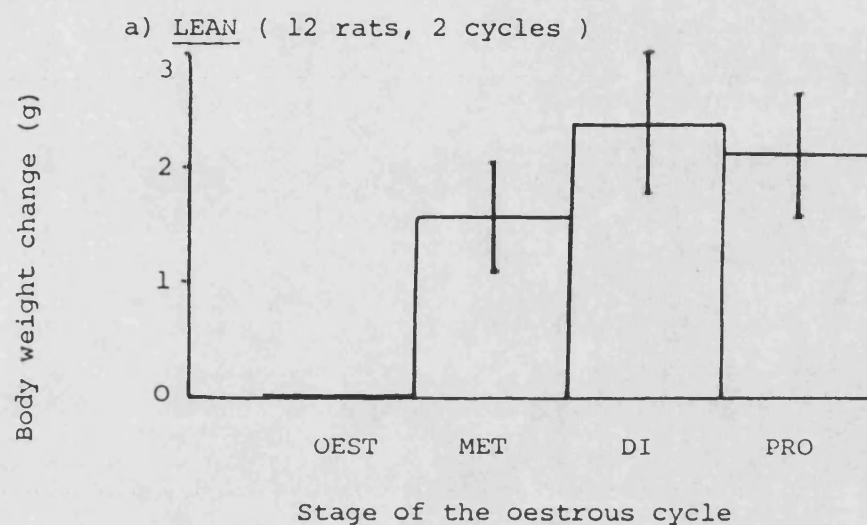
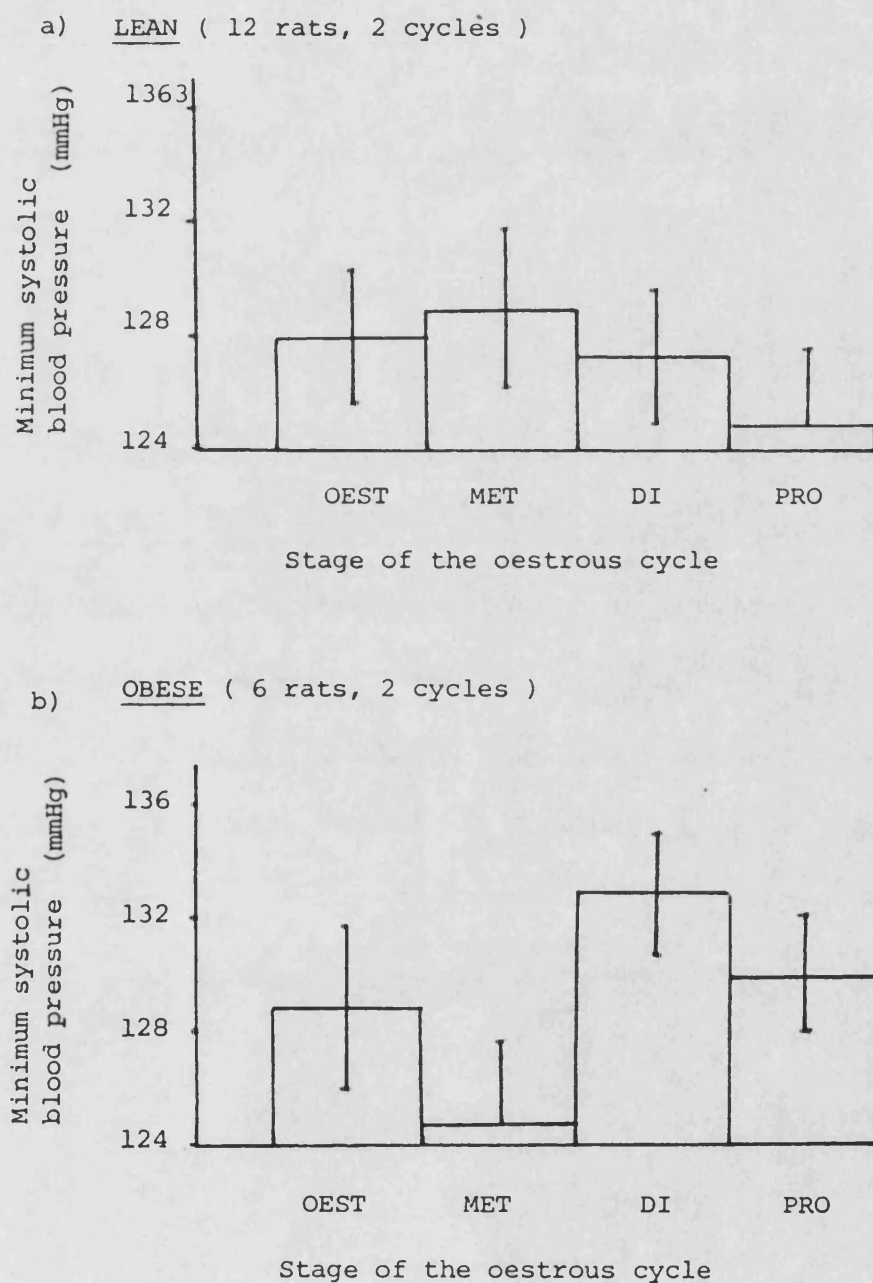


Fig.21 Minimum blood pressure readings obtained from lean and dietary obese rats at different stages of the oestrous cycle. All values mean \pm s.e.m.

Key: OEST = oestrus ; MET = metoestrus ; DI = dioestrus ;
PRO = pro-oestrus



3.4 THE EFFECTS OF DIETARY OBESITY ON PLASMA SODIUM AND POTASSIUM CONCENTRATION IN THE RAT.

3.4.1 Plasma sodium concentrations

Plasma sodium concentrations were determined after 1, 2, 3, 4, 8 and 20 weeks of study (laboratory chow with or without the varied palatable diet) (see Table 3.4(1)). After one week there was no significant difference between plasma sodium (Na^+) concentrations of lean (chow only) and dietary obese (chow plus palatable diet) rats. After 2 weeks, however, the dietary obese rats had a slightly greater plasma sodium concentration ($p < 0.05$). After 3 and 4 weeks the plasma sodium concentrations were greater in lean than in dietary obese rats (both $p < 0.001$). However, after 8 weeks there was again no significant difference between the groups. After 20 weeks the dietary obese rats had considerably greater plasma sodium concentrations than lean controls ($p < 0.001$). Plasma sodium concentrations were also determined for rats who had received the palatable diet in addition to laboratory chow for 4 weeks and then had the palatable diet withdrawn for 4 weeks, during which time they received chow only. Their mean plasma sodium concentration was found to be $151.05 \pm 2.36\text{mM}$ ($n=8$), which was not significantly different from either lean or dietary obese rats of the same age.

3.4.2 Plasma potassium concentrations

Plasma potassium (K^+) concentrations were measured for lean and dietary obese rats after 4, 8, and 20 weeks. There were no significant differences between the plasma potassium concentrations of lean and dietary obese rats after 4 or 8 weeks. However, after 20 weeks the dietary obese rats showed significantly higher potassium concentrations than lean rats ($p < 0.02$).

Table 3.4(i)

Concentrations (mM) of sodium (Na^+) in plasma samples from lean and dietary obese rats after 1, 2, 3, 4, 8, and 20 weeks. All values expressed as mean \pm s.e.m. for group.

	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
1 week	138.33 \pm 1.31 (n=6)	139.50 \pm 1.15 (n=6)	n.s.
2 weeks	143.67 \pm 1.23 (n=6)	146.33 \pm 1.05 (n=6)	<0.05
3 weeks	142.33 \pm 0.42 (n=6)	139.17 \pm 0.60 (n=6)	<0.001
4 weeks	144.42 \pm 1.05 (n=12)	141.36 \pm 0.83 (n=11)	<0.001
8 weeks	146.43 \pm 2.87 (n=6)	147.60 \pm 3.04 (n=6)	n.s.
20 weeks	134.80 \pm 1.56 (n=5)	148.00 \pm 2.13 (n=10)	<0.001

Table 3.4(ii)

Concentrations (mM) of potassium (K^+) in plasma samples from lean and dietary obese rats after 4, 8, and 20 weeks. All values expressed as mean \pm s.e.m. for group.

	<u>Lean</u>	<u>Dietary Obese</u>	<u>p*</u>
4 weeks	3.29 \pm 0.10 (n=9)	3.32 \pm 0.12 (n=3)	n.s.
8 weeks	4.00 \pm 0.19 (n=6)	4.09 \pm 0.31 (n=5)	n.s.
20 weeks	4.38 \pm 0.16 (n=5)	4.69 \pm 0.11 (n=9)	<0.02

* Significance calculated using Student's t-test

3.5 DIETARY SURVEY TO INVESTIGATE THE INTAKE OF CAFFEINE-CONTAINING BEVERAGES AMONG DIETERS AND NON-DIETERS.

3.5.1 General information about subjects

(For specimen Dietary Survey see Fig.3) 62 completed questionnaires were returned for analysis, of which 32 were from male subjects and 30 from female subjects. 24 (38.7%) of all subjects had dieted at some time ('dieters'), comprising 18 (60%) females ('female dieters') and 6 (18.8%) males ('male dieters'). It can be seen that dieting was greater among women than men, with over half of the women subjects having dieted at some time compared with less than one fifth of the men.

The age range of subjects overall was 19 to 63 years, with a mean of 28.70 ± 1.27 years (see Table 3.5(i) for details of ages of subjects). The mean age of the female subjects (30.90 ± 2.24 years) was significantly greater than for the male subjects (26.72 ± 1.22), $p < 0.001$.

3.5.2 Smoking habits (see Table 3.5(iii))

14 (22.6%) of the subjects were smokers, of whom 7 (23.3%) were women and 7 (21.9%) were men. Thus, the pattern of smoking habits was similar in men and women. The mean age (years) of smokers (35.57 ± 3.20) was significantly greater than that of non-smokers (26.75 ± 1.23), $p < 0.001$. This was true for both female smokers (39.29 ± 5.54) versus female non-smokers (28.35 ± 2.19) and male smokers (31.86 ± 3.03) versus male non-smokers (25.28 ± 1.19), both $p < 0.001$.

The mean number of cigarettes smoked per day (for the 10 smokers who reported the number smoked per day) was 11.65 ± 2.34 . There was no significant difference between the number of cigarettes smoked daily by male ($n=4$) and female ($n=6$) subjects, 13.25 ± 8.30 and 10.53

± 2.98 , respectively. There was also no significant difference between the number of cigarettes smoked daily by dieters ($n=4$) and non-dieters ($n=6$), 9.00 ± 3.34 and 13.42 ± 3.22 , respectively. The number of cigarettes smoked daily was not altered when dieting for any of the subjects.

3.5.3 Body Mass Index (see Table 3.5(11))

Body Mass Index (BMI) for all subjects ranged from 16.3 to 30.7 a mean of 22.20 ± 0.34 . Dieters had a significantly greater BMI than non-dieters (23.30 ± 0.62 and 21.51 ± 0.36), $p<0.001$. This held true also for females: dieters (22.47 ± 0.63) and non-dieters (20.75 ± 0.72), $p<0.001$, and also for males: dieters (25.82 ± 1.17) and non-dieters (21.85 ± 0.40), $p<0.001$. The male dieters were the only group which showed a BMI in the Grade 1 obesity category (Garrow, 1988), all other groups fell into the Grade 0 category. There was no significant difference between the BMI of smokers (21.99 ± 0.86) and non-smokers (22.26 ± 0.37). There was also no difference between the BMI of male (22.60 ± 0.47) and female (21.78 ± 0.49) subjects, although the males tended to have a greater mean BMI.

The BMI of groups of subjects were analysed according to the number of cups of coffee consumed daily. The results were as follows: 0 cups ($n=9$), BMI= 22.34 ± 0.33 ; 1-2 cups ($n=17$), BMI= 21.02 ± 0.46 ; 3-4 cups ($n=18$), BMI= 22.53 ± 0.76 ; 5,6,7 cups ($n=11$), BMI= 23.32 ± 0.75 ; 8+ cups ($n=7$), BMI= 22.29 ± 1.54 . Although there was no obvious trend in the results, subjects consuming 1 or 2 cups of coffee per day had a significantly lower BMI than non-coffee drinkers ($p<0.05$) and also lower than that of subjects who consumed 3 or more cups of coffee daily (22.72 ± 0.52) ($p<0.001$).

Table 3.5(i)

Ages (years) of subjects according to sex, dieting history and smoking habits.

a) Dieting history

	<u>Dieter</u>	<u>Non-dieter</u>	<u>p*</u>
Total	28.96 \pm 2.32 (n=24)	28.61 \pm 1.49 (n=38)	n.s.
Female	29.33 \pm 2.98 (n=18)	33.25 \pm 3.41 (n=12)	n.s.
Male	27.83 \pm 2.91 (n=6)	26.46 \pm 1.36 (n=26)	n.s.

b) Smoking habits

	<u>Smoker</u>	<u>Non-smoker</u>	<u>p*</u>
Total	35.57 \pm 3.20 (n=14)	26.75 \pm 1.23 (n=48)	<0.001
Female	39.29 \pm 5.54 (n=7)	28.35 \pm 2.19 (n=23)	<0.001
Male	31.86 \pm 3.03 (n=7)	25.28 \pm 1.19 (n=25)	<0.001

Table 3.5(ii)

Body mass index (W/H^2) of subjects according to sex, dieting history and smoking habits.

a) Dieting history

	<u>Dieter</u>	<u>Non-dieter</u>	<u>p*</u>
Total	23.30 \pm 0.62 (n=24)	21.51 \pm 0.36 (n=38)	<0.001
Female	22.47 \pm 0.63 (n=18)	20.75 \pm 0.72 (n=12)	<0.01
Male	25.82 \pm 1.17 (n=6)	21.85 \pm 0.40 (n=26)	<0.001

b) Smoking habits

	<u>Smokers</u>	<u>Non-smokers</u>	<u>p*</u>
Total	21.99 \pm 0.86 (n=14)	22.26 \pm 0.37 (n=48)	n.s.
Female	22.14 \pm 1.70 (n=7)	21.67 \pm 0.42 (n=23)	n.s.
Male	21.83 \pm 0.52 (n=7)	22.81 \pm 0.58 (n=25)	n.s.

All values expressed as mean \pm s.e.m.

* Significance levels calculated using Student's t-test.

Table 3.5(iii)

Number of cigarettes smoked daily (mean \pm s.e.m. for the 10 out of 14 total smokers who stated the number of cigarettes smoked daily) according to sex and dieting history.

<u>Category of smoker (n)</u>	<u>Number of cigarettes smoked daily</u>
Total (10)	11.65 \pm 2.34
Male (4)	13.25 \pm 8.30
Female (6)	10.53 \pm 2.98
Dieter (4)	9.00 \pm 3.34
Non-dieter (6)	13.42 \pm 3.22
Female dieter (3)	8.67 \pm 4.70
Male dieter (1)	10.00
Female non-dieter (3)	12.50 \pm 4.33
Male non-dieter (3)	14.33 \pm 5.67

Note: Values for dieters apply both when dieting and not dieting, as the number of cigarettes smoked daily was not altered by dieting.

3.5.4 Coffee consumption

a) General (see Table 3.5(v))

Coffee consumption ranged from 0 to 16 cups daily, with a mean value for all subjects of 3.55 ± 0.38 cups per day. There was no significant difference in coffee consumption between male and female subjects, with males consuming 3.56 ± 0.45 cups per day and females 3.53 ± 0.62 cups per day. If one assumes an 'average' cup of coffee to contain approximately 85mg caffeine (see Appendix 3), then the subjects had a mean daily caffeine intake of approximately 300mg (from coffee).

b) The effects of dieting on coffee consumption

For 6 (25%) dieters coffee intake increased when dieting, while one (4.2%) dieter decreased and 17 (70.8%) did not alter consumption (see Tables 3.5(v)a) and 3.5(vi)a)). There was no significant difference between daily coffee consumption among total dieters, when they were not dieting (4.08 ± 0.67) and non-dieters (3.21 ± 0.44). However, when male dieters only were considered, they consumed significantly more cups of coffee per day, when not dieting (4.83 ± 0.79) than non-dieters (3.27 ± 0.51), $p < 0.05$. When dieting, dieters consumed significantly more coffee (4.58 ± 0.73) than non-dieters, $p < 0.01$. This was mainly due to the greater coffee consumption of male dieters when dieting (5.83 ± 1.19) compared with male non-dieters (3.27 ± 0.51), $p < 0.001$. Although coffee consumption was raised in female dieters while dieting (4.17 ± 0.89) this was not significantly higher than for the female non-dieters (3.08 ± 0.88) intake. Thus, there is clear evidence that some dieters do increase their coffee consumption when dieting, this being more prominent in men than women.

c) Smoking habits and coffee consumption

Smokers drank significantly more cups of coffee each day than non-smokers (4.64 ± 0.87 and 3.23 ± 0.41 , respectively), $p < 0.001$ (see Table 3.5(v)b)). This difference was due mainly to the greater amount of coffee consumed daily by male smokers (5.71 ± 1.08 cups) compared with male non-smokers (2.96 ± 0.43 cups), $p < 0.001$. There was no significant difference between daily coffee consumption of female smokers (3.57 ± 1.31 cups) and female non-smokers (3.52 ± 0.72 cups). There was a significant correlation between the number of cigarettes smoked daily and the number of cups of coffee consumed daily ($r = 0.6826$, $n = 10$, $p < 0.05$), (see Fig.22). When combined coffee and tea consumption (total cups per day) was investigated this too was found to correlate significantly with the number of cigarettes smoked daily ($r = 0.7344$, $n = 10$, $p < 0.02$), (see Fig.23).

3.5.5 Tea consumption

Tea consumption ranged from 0 to 8 cups daily, with a mean value for all subjects of 1.82 ± 0.26 cups per day (see Table 3.5(iv)). Overall, women drank twice as many cups of tea per day as men (2.40 ± 0.39 and 1.28 ± 0.31 , respectively), $p < 0.001$. Female dieters drank approximately four times as much tea per day as male dieters, both when not dieting (2.61 ± 0.49 and 0.67 ± 0.33) and when dieting (2.83 ± 0.53 and 0.67 ± 0.33) both $p < 0.001$. However, in male and female non-dieters tea consumption was not significantly different (1.42 ± 0.36 and 2.50 ± 0.78 cups per day, respectively). Tea consumption was similar in dieters, when not dieting, (2.12 ± 0.41) and non-dieters (1.63 ± 0.33) and tea consumption did not alter significantly among dieters overall, when they were dieting (2.29 ± 0.44).

Table 3.5(iv)

Number of cups of tea normally consumed daily by subjects (when not dieting) according to sex, dieting history and smoking habits.

a) Dieting history

	<u>Dieters</u>	<u>Non-dieters</u>	<u>p*</u>
Total	2.12 \pm 0.40 (n=24)	1.63 \pm 0.33 (n=38)	n.s.
Female	2.61 \pm 0.49 (n=18)	2.50 \pm 0.78 (n=12)	n.s.
Male	0.67 \pm 0.33 (n=6)	1.42 \pm 0.36 (n=26)	n.s.

b) Smoking habits

	<u>Smoker</u>	<u>Non-smoker</u>	<u>p*</u>
Total	2.07 \pm 0.53 (n=14)	1.75 \pm 0.29 (n=48)	n.s.
Female	3.00 \pm 0.76 (n=7)	2.22 \pm 0.46 (n=23)	n.s.
Male	1.14 \pm 0.59 (n=7)	1.32 \pm 0.36 (n=25)	n.s.

Table 3.5(v)

Number of cups of coffee normally consumed daily by subjects (when not dieting) according to sex, dieting history and smoking habits.

a) Dieting history

	<u>Dieters</u>	<u>Non-dieters</u>	<u>p*</u>
Total	4.08 \pm 0.67 (n=24)	3.21 \pm 0.44 (n=38)	n.s.
Female	3.83 \pm 0.86 (n=18)	3.08 \pm 0.88 (n=12)	n.s.
Male	4.83 \pm 0.79 (n=6)	3.27 \pm 0.51 (n=26)	<0.05

b) Smoking habits

	<u>Smokers</u>	<u>Non-smokers</u>	<u>p*</u>
Total	4.64 \pm 0.87 (n=14)	3.23 \pm 0.41 (n=48)	<0.001
Female	3.57 \pm 1.31 (n=7)	3.52 \pm 0.72 (n=23)	n.s.
Male	5.71 \pm 1.08 (n=7)	2.96 \pm 0.43 (n=25)	<0.001

All values expressed as mean \pm s.e.m. for group.

* Significance calculated using Student's t-test

Fig.22 Diagram illustrating correlation between the number of cigarettes smoked daily and the number of cups of coffee consumed daily among subjects who reported the number of cigarettes smoked daily (n = 10).

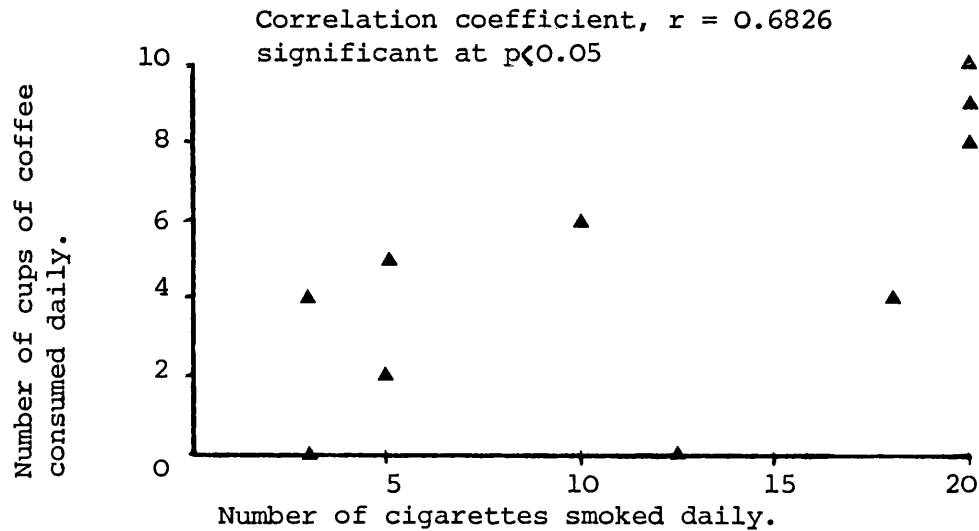


Fig. 23 Diagram illustrating correlation between the number of cigarettes smoked daily and the number of cups of tea plus coffee consumed daily among subjects who reported the number of cigarettes smoked daily (n = 10).

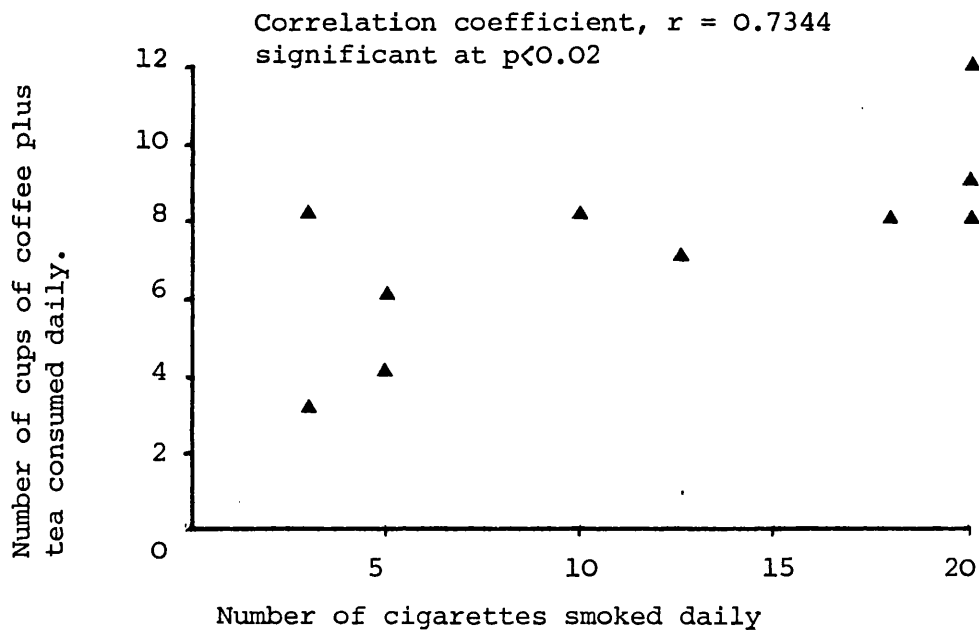


Table 3.5(vi)

Number of cups of tea and coffee consumed daily by male and female subjects according to dieting status

	<u>Male</u>	<u>Female</u>	<u>p*</u>
a) <u>Coffee</u>			
Non-dieters	3.27 \pm 0.51 (n=26)	3.08 \pm 0.88 (n=12)	n.s.
Dieters			
i) dieting	5.83 \pm 1.19 (n=6)	4.17 \pm 0.89 (n=18)	n.s.
ii)not dieting	4.83 \pm 0.79 (n=6)	3.83 \pm 0.86 (n=18)	n.s.
b) <u>Tea</u>			
Non-dieters	1.42 \pm 0.36 (n=26)	2.50 \pm 0.78 (n=12)	n.s.
Dieters			
i) dieting	0.67 \pm 0.33 (n=6)	2.83 \pm 0.53 (n=18)	<0.001
ii)not dieting	0.67 \pm 0.33 (n=6)	2.61 \pm 0.49 (n=18)	<0.001

Table 3.5(vii)

Normal daily alcohol intake ("standard drinks") of male and female subjects according to dieting history.

	<u>Male</u>	<u>Female</u>	<u>p*</u>
Total	1.69 \pm 0.33 (n=32)	0.98 \pm 0.19 (n=30)	<0.001
Dieters	2.17 \pm 0.48 (n=6)	1.07 \pm 0.24 (n=18)	<0.01
Non-dieters	1.58 \pm 0.39 (n=26)	0.85 \pm 0.31 (n=12)	<0.05

All values expressed as mean \pm s.e.m. for group.

* Significance calculated using Student's t-test.

3.5.6 Consumption of cola-type drinks

Only 7 subjects reported drinking any cola drinks. These subjects were all male (3 dieters, 4 non-dieters). Of the 3 dieters, one reported increased cola consumption when dieting, one reported decreased consumption and one reported unchanged consumption.

3.5.7 Consumption of alcoholic beverages

Reported alcohol consumption was converted to 'standard drinks', as described by the Health Education Council in the publication "That's the Limit" (Health Education Council, 1987). Daily intake of alcohol ranged from 0 to 8 'standard drinks' (see Table 3.5(vii)), with a mean value of 1.35 ± 0.20 'standard drinks' per day.

Alcohol consumption among smokers was less than for non-smokers (0.85 ± 0.29 and 1.50 ± 0.24 'standard drinks' per day), $p < 0.001$. Male subjects consumed significantly more alcohol than female subjects. This held true for dieters, when not dieting, (male, 2.17 ± 0.48 and female, 1.07 ± 0.24), $p < 0.01$; non-dieters (male, 1.58 ± 0.39 and female, 0.85 ± 0.31), $p < 0.05$; and total subjects (male, 1.69 ± 0.33 and female, 0.98 ± 0.19), $P < 0.001$. When dieting, dieters reduced their alcohol intake (see Table 3.5(viii) from 1.35 ± 0.23 to 0.63 ± 0.16 , $p < 0.001$. This held true for both female dieters (1.07 ± 0.24 and 0.45 ± 0.14), $p < 0.01$ and also for male dieters (2.17 ± 0.48 and 1.17 ± 0.48), $p < 0.05$. There was a weak, though significant correlation between BMI and alcohol intake (expressed as 'standard drinks' per day') for all subjects ($r = 0.2788$, $n = 62$, $p < 0.05$). When dieters (not dieting) only were considered, this correlation was stronger ($r = 0.6773$, $n = 24$, $p < 0.001$), see Fig.24. There was also a significant correlation between BMI and alcohol consumption for female dieters only (not dieting) ($r = 0.6105$, $n = 18$, $p < 0.01$).

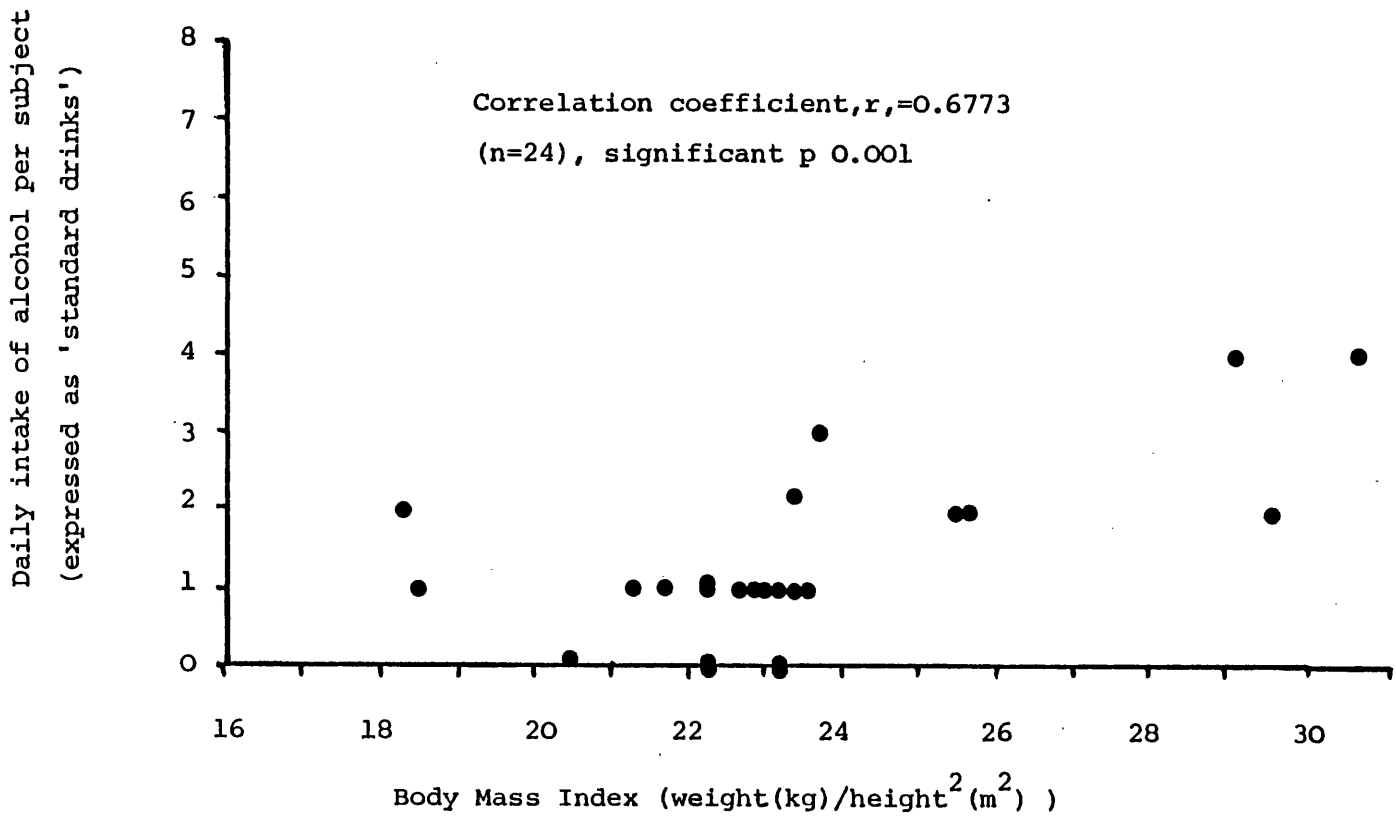
Table 3.5(viii)

The effects of dieting on alcohol consumption ("standard drinks" per day) of dieters. All values expressed as mean \pm s.e.m. for group.

	<u>Dieting</u>	<u>Not dieting</u>	<u>p*</u>
Total (n=24)	0.63 \pm 0.16	1.35 \pm 0.23	<0.001
Female (n=18)	0.45 \pm 0.14	1.07 \pm 0.24	<0.01
Male (n=6)	1.17 \pm 0.48	2.17 \pm 0.48	<0.05

* Significance calculated using Student's t-test.

Fig.24 Correlation between Body Mass Index and daily alcohol intake (when not dieting) amongst dieters. (n=24)



3.6 THE EFFECTS OF CAFFEINE (25MG/KG AND 50MG/KG), GIVEN WITH AND WITHOUT A PALATABLE DIET, IN FEMALE WISTAR RATS.

Abbreviations for the groups used in this section are as follows:

L = 4 weeks lean controls (untreated, chow-fed)

D = 4 weeks dietary obese (untreated, chow plus palatable diet)

LC25 = 4 weeks lean + caffeine 25mg/kg daily

DC25 = 4 weeks dietary obese + caffeine 25mg/kg daily

LC50 = 4 weeks lean + caffeine 50mg/kg daily

DC50 = 4 weeks dietary obese + caffeine 50mg/kg daily

Data for groups L and D have already been presented in section 3.1, as results for lean and dietary obese rats after 4 weeks of study.

The time periods (weeks) referred to in this section are the length of time from the start of the study during which the rats have received drug and/or dietary manipulation.

3.6.1 The effects on body weight (see Table 3.6(1) and Fig.25)

There were no significant differences between the weights of the different groups at the start of the week 1. After the 4 weeks of the study LC25 had gained significantly less weight than L ($+23.33 \pm 2.38\text{g}$ and $+31.58 \pm 2.63\text{g}$, respectively), $p < 0.01$ and LC50 ($+38.50 \pm 2.32$) had gained significantly more weight than L, $p < 0.05$. Both DC25 ($+84.67 \pm 7.20\text{g}$) and DC50 ($+84.75 \pm 4.92\text{g}$) had gained significantly more weight than D ($+66.25 \pm 4.46\text{g}$) during the 4 weeks of the study, both $p < 0.001$. All dietary obese groups gained significantly more weight than their lean counterparts, these differences being significant at the $p < 0.001$ level.

Table 3.6(1)

Weights (g) of groups of rats throughout the 'caffeine study'. All values expressed as mean \pm s.e.m. for each group (n=12 for each group). For key to abbreviations for groups used see section 3.6.

<u>Group</u>	<u>Start of Week 1</u>	<u>Start of Week 2</u>	<u>Start of Week 3</u>	<u>Start of Week 4</u>	<u>End of Week 4</u>
L	211.42 \pm 3.12	226.25 \pm 3.18	230.42 \pm 3.54	238.75 \pm 4.18	243.00 \pm 4.10
D	209.00 \pm 3.54	224.83 \pm 4.15	246.92 \pm 4.86	258.92 \pm 5.47	275.25 \pm 6.80
LC25	209.17 \pm 2.11	218.83 \pm 2.31	225.17 \pm 2.70	229.50 \pm 3.01	232.42 \pm 3.73
DC25	213.83 \pm 1.95	235.67 \pm 2.27	259.00 \pm 3.50	275.08 \pm 3.82	298.50 \pm 6.71
LC50	213.25 \pm 2.63	229.25 \pm 4.00	233.00 \pm 4.35	244.83 \pm 3.98	251.75 \pm 4.22
DC50	212.00 \pm 1.39	238.00 \pm 2.74	259.67 \pm 3.86	273.92 \pm 4.04	296.75 \pm 4.62

3.6.2 The effects on minimum systolic blood pressure (see Fig.26)

As can be seen from Table 3.6(ii), the minimum systolic blood pressure values (mmHg) for the different groups were dissimilar at the start of week 1, which made the use of the absolute values questionable. Therefore, the changes in blood pressure over the 4 weeks of the study were used. The increase in blood pressure of group D ($+18.50 \pm 4.11$ mmHg) was significantly greater than for L ($+7.67 \pm 3.50$ mmHg), $p < 0.001$. Considering the effects of caffeine: there was a small decrease in blood pressure for LC50 (-3.25 ± 3.66 mmHg) during the study, thus the change in blood pressure for LC50 was significantly different compared to that for L ($p < 0.001$). The increase in blood pressure of DC50 ($+7.50 \pm 3.22$ mmHg) was significantly less than that of D ($+18.50 \pm 4.11$ mmHg) $p < 0.001$. However, DC25 showed an increase in blood pressure ($+21.67 \pm 3.56$ mmHg) slightly greater than that of D (palatable diet alone), although this difference was not statistically significant.

Thus, in summary, all dietary obese groups showed significantly greater increases in minimum systolic blood pressure than their lean counterparts. While caffeine 25mg/kg produced no significant effects upon the change in blood pressure over the 4 week period in either lean (LC25) or dietary obese (DC25) rats compared to L and D, respectively the higher dose of 50mg/kg reduced the increase in blood pressure seen with time in both lean and dietary obese rats to such an extent that the change in blood pressure of DC50 was similar to that of L, while a small reduction in blood pressure over the 4 weeks was seen for LC50.

Table 3.6(ii)

Minimum systolic blood pressure values (mmHg) of the groups of rats during the study. All values expressed as mean \pm s.e.m. for group (n=12 for all groups).

<u>Group</u>	<u>Start of Week 1</u>	<u>End of Week 4</u>	<u>Change over 4 weeks</u>
L	106.67 \pm 1.78	114.33 \pm 3.34	+7.67 \pm 3.50
D	106.67 \pm 3.51	125.42 \pm 3.42	+18.50 \pm 4.11
LC25	120.67 \pm 3.28	122.92 \pm 3.26	+2.25 \pm 4.72
DC25	128.67 \pm 2.24	150.33 \pm 3.88	+21.67 \pm 3.56
LC50	124.75 \pm 4.25	121.50 \pm 3.84	-3.25 \pm 3.66
DC50	128.42 \pm 3.73	135.92 \pm 4.28	+7.50 \pm 3.22

Table 3.6(iii)

'In vivo' heart rate (beats per minute) of the groups of rats during the study. All values expressed as mean \pm s.e.m. for group (n=12 for all groups).

<u>Group</u>	<u>Start of Week 1</u>	<u>End of Week 4</u>	<u>Change over 4 weeks</u>
L	380.67 \pm 10.21	389.17 \pm 7.23	+8.15 \pm 9.36
D	372.04 \pm 6.68	416.25 \pm 6.96	+44.21 \pm 10.03
LC25	375.67 \pm 9.74	347.32 \pm 7.92	-28.35 \pm 7.60
DC25	355.33 \pm 8.67	383.69 \pm 7.13	+28.36 \pm 9.18
LC50	335.98 \pm 8.34	353.75 \pm 8.21	+17.77 \pm 10.24
DC50	356.67 \pm 9.23	377.96 \pm 9.24	+21.29 \pm 12.22

For abbreviations of groups used see section 3.6.

Fig.25 The effects of diet and caffeine on the increase in body weight occurring during the 4 weeks of the study.

All values expressed as mean \pm s.e.m.)

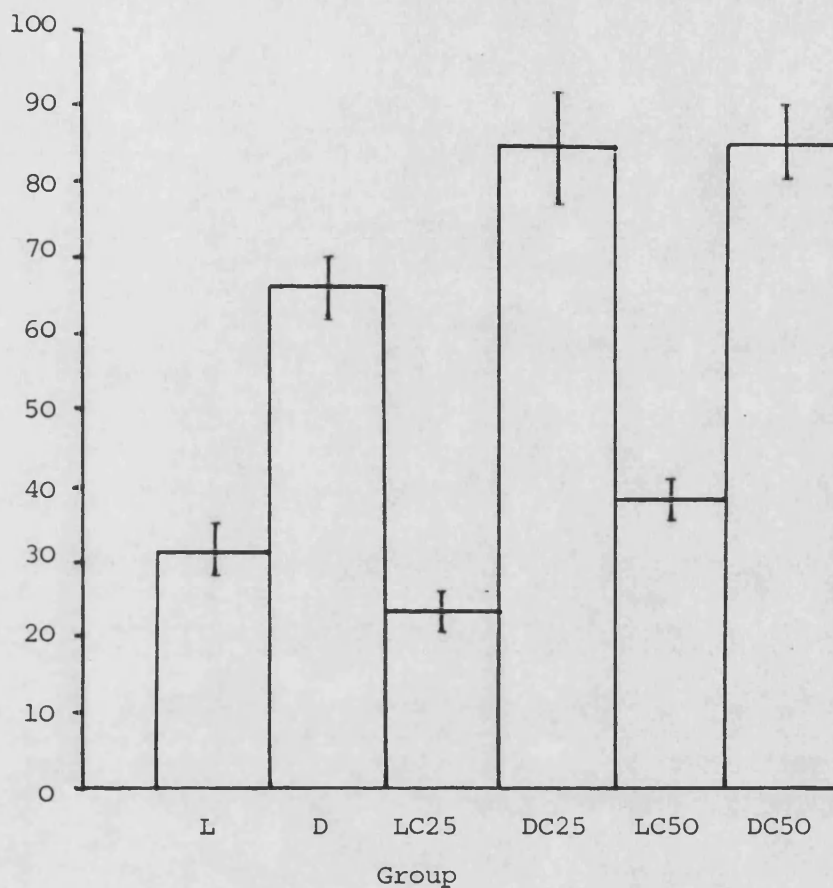
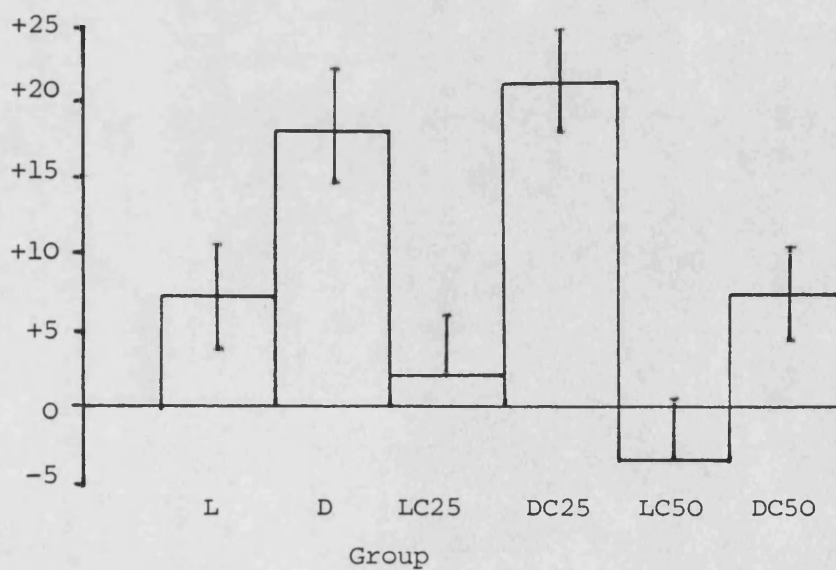


Fig.26 The effects of diet and caffeine on the change in systolic blood pressure occurring during the 4 weeks of the study. All values expressed as mean \pm s.e.m.



3.6.3 The effects on 'in vivo' heart rate (see Table 3.6(iii))

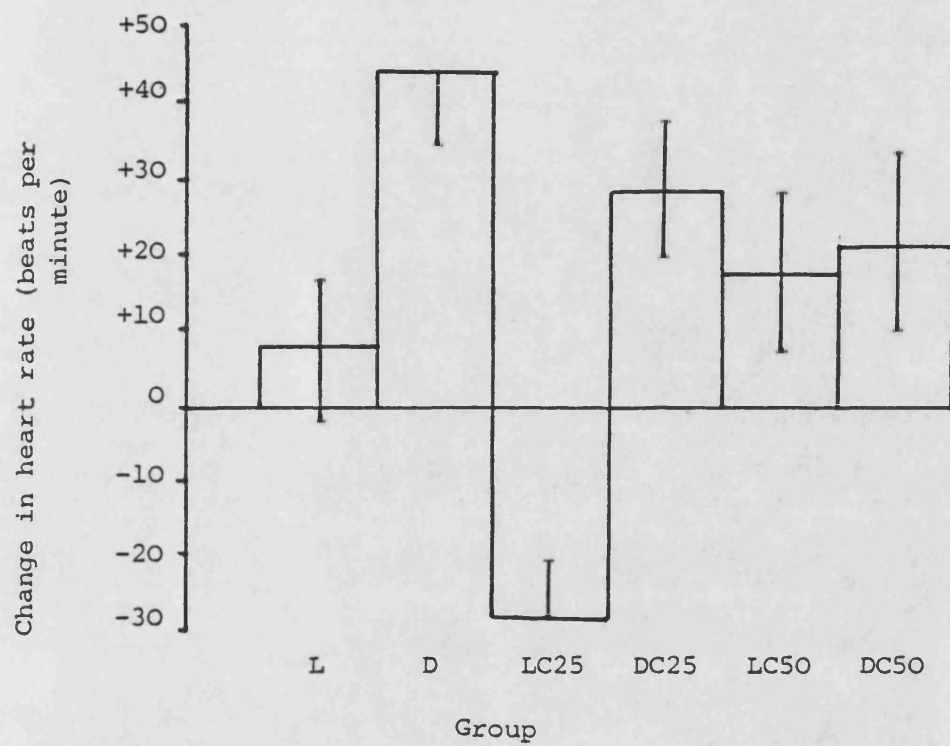
As with the minimum systolic blood pressure data (see Table 3.6(ii)), the starting values for mean heart rate were dissimilar for the different groups (see Table 3.6(iii)) and therefore the changes in rate during the 4 weeks of the study were used in preference to the absolute values (see also Fig.27) . At the end of the 4 weeks of the study greater increases in heart rate were seen for all the dietary obese groups compared with their lean counterparts. However, this was only significant for group D ($+44.21 \pm 10.03$ beats per minute) compared with L ($+8.15 \pm 9.36$ beats per minute) and for DC25 ($+28.36 \pm 9.18$) compared with LC25 (-28.35 ± 7.60), both $p < 0.001$. Although the change in heart rate of DC50 ($+21.29 \pm 12.22$) was greater than that of LC50 ($+17.77 \pm 10.24$), the difference did not reach significance.

Considering the effects of caffeine: the value for LC25 (-28.35 ± 7.60) was significantly lower than that for L ($+8.15 \pm 9.36$), $p < 0.001$, while the value for LC50 was similar to that for L (in fact showing a small, non-significant elevation). In the dietary obese rats both 25mg/kg and 50mg/kg caffeine resulted in an attenuation of the increase in heart rate seen with time (compared with the untreated dietary obese group, D), although this effect was only significant with the higher dose ($p < 0.01$).

Fig.27

Effect of diet and caffeine on the change in 'in vivo' heart rate occurring during the 4 weeks of the study.

All values expressed as mean \pm s.e.m.



3.6.4 Caffeine and food intake

Food intake (for 24 hour periods) was monitored at various times throughout the study. Intake of laboratory chow (in g) was : L, 16.57 \pm 0.30 (n=9); LC25, 16.01 \pm 0.19 (n=10); LC50, 17.68 \pm 0.47 (n=5) (n=number of readings). Thus food intake was increased with the high dose of caffeine, significant at $p < 0.001$. The slight decrease in daily food intake with the low dose of caffeine did not reach statistical significance. Food intake of the groups offered the palatable diet was monitored once only (during week 4). From these data the nutrient content of the foods eaten was calculated and can be seen below in Table 3.6(iv).

Table 3.6(iv)

Daily nutrient intake per rat (in grams unless otherwise stated)

<u>Group</u>	<u>Fat</u>	<u>Carbo-</u> <u>hydrate</u>	<u>Protein</u>	<u>Fibre</u>	<u>Sodium</u> <u>(mg)</u>	<u>Energy</u> <u>(kcal)</u>
D	2.01	15.19	2.03	0.87	170.23	82.24
DC25	1.88	13.42	1.61	0.79	182.67	73.34
DC50	2.25	14.46	1.98	0.93	191.44	81.80

Although no statistical analysis is possible for these results, they give an indication of the quantities consumed by the dietary obese groups of rats and there appears to be very little difference between the groups. Certainly, there is no indication of the type of increase in food consumption seen in LC50 (high dose caffeine, lean).

3.6.5 'In vitro' studies: the isolated perfused heart

The baseline beating rate and baseline contractility (under 2g basal tension) were recorded for isolated perfused heart preparations from rats from each of the groups (L, D, LC25, DC25, LC50, DC50) at the end of the 4-week study. The responses to three doses of noradrenaline (beating rate, contractility and arrhythmias) and two doses of acetylcholine (duration of cardiac arrest and recovery time) were also studied for each heart preparation. The doses of noradrenaline used were 5×10^{-11} mole, 5×10^{-10} mole and 5×10^{-9} mole and the doses of acetylcholine used were 5×10^{-9} mole and 5×10^{-8} mole.

a) Beating rate (see Table 3.6(v) and Fig.28)

Baseline 'in vitro' beating rates were faster for hearts from dietary obese rats than for hearts from lean rats, though this difference was only significant for hearts from DC50 (262.22 ± 6.13 beats per minute) compared with LC50 (234.09 ± 9.31 beats per minute), $p < 0.001$.

Administration of caffeine resulted in a dose-dependent reduction in baseline beating rates in hearts from the lean groups, although this was only significant for LC50 (234.09 ± 9.31) compared with LC25 and L (260.00 ± 5.84 and 271.67 ± 6.29 , respectively), $p < 0.001$. Beating rate of hearts from LC25 were not significantly less than those of hearts from L. Chronic administration of caffeine did not affect the 'in vitro' beating rate of hearts from the dietary obese rats.

Table 3.6(v)

The effects of 4 weeks of daily caffeine administration on the baseline beating rate (beats per minute) of the isolated perfused hearts from groups of rats and the effects of three doses of noradrenaline (added 'in vitro') on this rate. All values expressed as mean \pm s.e.m. for group.

<u>Group</u>	<u>Baseline</u>	<u>Increase in beating rate induced by dose of</u>		
	<u>beating rate</u>	<u>noradrenaline (beats per minute)</u>		
		<u>5×10^{-11} mole</u>	<u>5×10^{-10} mole</u>	<u>5×10^{-9} mole</u>
L	271.67 \pm 6.29	17.78 \pm 2.37	58.33 \pm 6.12	88.33 \pm 11.02
(n=9)				
D	262.22 \pm 10.35	20.56 \pm 6.94	53.33 \pm 14.84	78.89 \pm 13.74
(n=9)				
LC25	260.00 \pm 5.84	15.91 \pm 4.90	36.36 \pm 10.02	67.73 \pm 12.22
(n=11)				
DC25	249.00 \pm 9.96	21.67 \pm 5.98	55.00 \pm 10.50	95.00 \pm 14.12
(n=12)				
LC50	234.09 \pm 9.31	19.55 \pm 6.01	54.55 \pm 8.54	88.18 \pm 10.90
(n=11)				
DC50	262.22 \pm 6.13	22.78 \pm 3.13	55.56 \pm 6.26	77.22 \pm 11.91
(n=9)				

Abbreviations used for groups:

L = 4 weeks lean (chow-fed), untreated

D = 4 weeks dietary obese (chow plus palatable diet), untreated

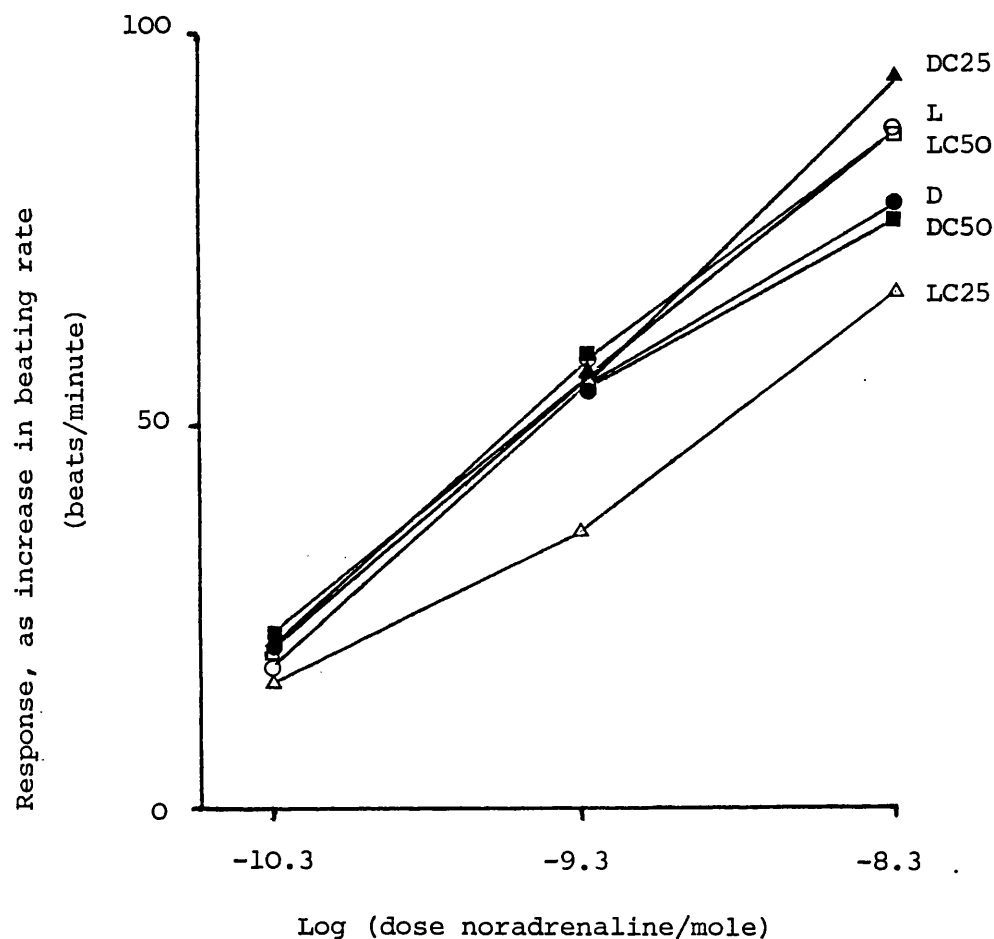
LC25 = 4 weeks lean, caffeine 25mg/kg daily

DC25 = 4 weeks dietary obese, caffeine 25mg/kg daily

LC50 = 4 weeks lean, caffeine 50mg/kg daily

DC50 = 4 weeks dietary obese, caffeine 50mg/kg daily

Fig.28 The effect of noradrenaline upon beating rate of isolated perfused hearts from lean and dietary obese rats and the changes induced by chronic treatment (4 weeks) with two doses of caffeine. All values shown as mean, error bars omitted, for s.e.m. values see section 3.6.5 a)i).



Key

L = Lean, 4 weeks, untreated	○ — ○ (n=9)
D = Dietary obese, 4 weeks, untreated	● — ● (n=9)
LC25 = Lean, caffeine 25mg/kg	△ — △ (n=11)
DC25 = Dietary obese, caffeine 25mg/kg	▲ — ▲ (n=12)
LC50 = Lean, caffeine 50mg/kg	□ — □ (n=11)
DC50 = Dietary obese, caffeine 50mg/kg	■ — ■ (n=9)

Considering the increase in rate induced by 5×10^{-11} mole noradrenaline: there were no significant differences between data from either dietary obese and lean groups or between the caffeine treated and untreated groups. The hearts from the dietary obese groups did tend to give larger responses than hearts from their lean counterparts, though these differences were not significant. Considering the increase in rate induced by 5×10^{-10} mole noradrenaline: hearts from all the dietary obese groups gave similar responses. Hearts from the lean group, which had received 25mg/kg caffeine (LC25), gave significantly lower responses ($+36.36 \pm 10.02$ beats per minute) than hearts from both untreated lean rats (L) ($+58.33 \pm 6.12$) and hearts from lean rats which had received the high dose of caffeine (LC50) (54.55 ± 8.54), both at $p < 0.01$. Lastly, considering the increase in rate induced by 5×10^{-9} mole noradrenaline. Hearts from DC25 gave significantly greater responses than hearts from LC25, $p < 0.01$, though no other significant differences were observed with this dose. The hearts from LC25 gave the smallest responses to all doses of noradrenaline, which may be of interest as this group of rats also showed a considerable fall in 'in vivo' heart rate during the 4 weeks of caffeine administration.

b) Contractility under 2g tension (see Table 3.6(vi) and Fig.29)

The hearts from all the caffeine-treated groups (LC25, DC25, LC50, DC50) had significantly greater baseline contractility than hearts from the corresponding untreated groups (L and D). No significant differences were found between the baseline contractility of the different drug-treated groups, although the hearts from the obese groups tended to have lower contractility than their lean counterparts. In addition, the 50mg/kg treated groups tended to have

greater contractility than their respective 25mg/kg treated counterparts. Hearts from the dietary obese group (D) gave significantly greater responses than those from lean animals (L) to all three doses of noradrenaline (all at $p < 0.001$). However, the responses to all three doses of noradrenaline were significantly reduced in hearts from dietary obese rats which had received caffeine (DC25 and DC50) than those for hearts from untreated obese animals (D), all at $p < 0.001$. Similarly, for the hearts from lean rats which had received caffeine (LC25 and LC50), the responses to the two higher doses of noradrenaline were significantly smaller than those of hearts from untreated lean controls (L), all at $p < 0.01$ (except for the 5×10^{-9} mole dose in hearts from LC25 which did not reach statistical significance).

c) The occurrence of arrhythmias after doses of noradrenaline

The number of arrhythmias (ectopic beats and missed beats) which occurred in hearts from the different groups (L, D, LC25, DC25, LC50, DC50) after each of the three doses of noradrenaline were analysed using the non-parametric Mann-Whitney U-test (see Table 3.6(vii) for the number of arrhythmias occurring in each preparation). No significant differences were found, between the groups, regarding the number of arrhythmias occurring after any dose of noradrenaline.

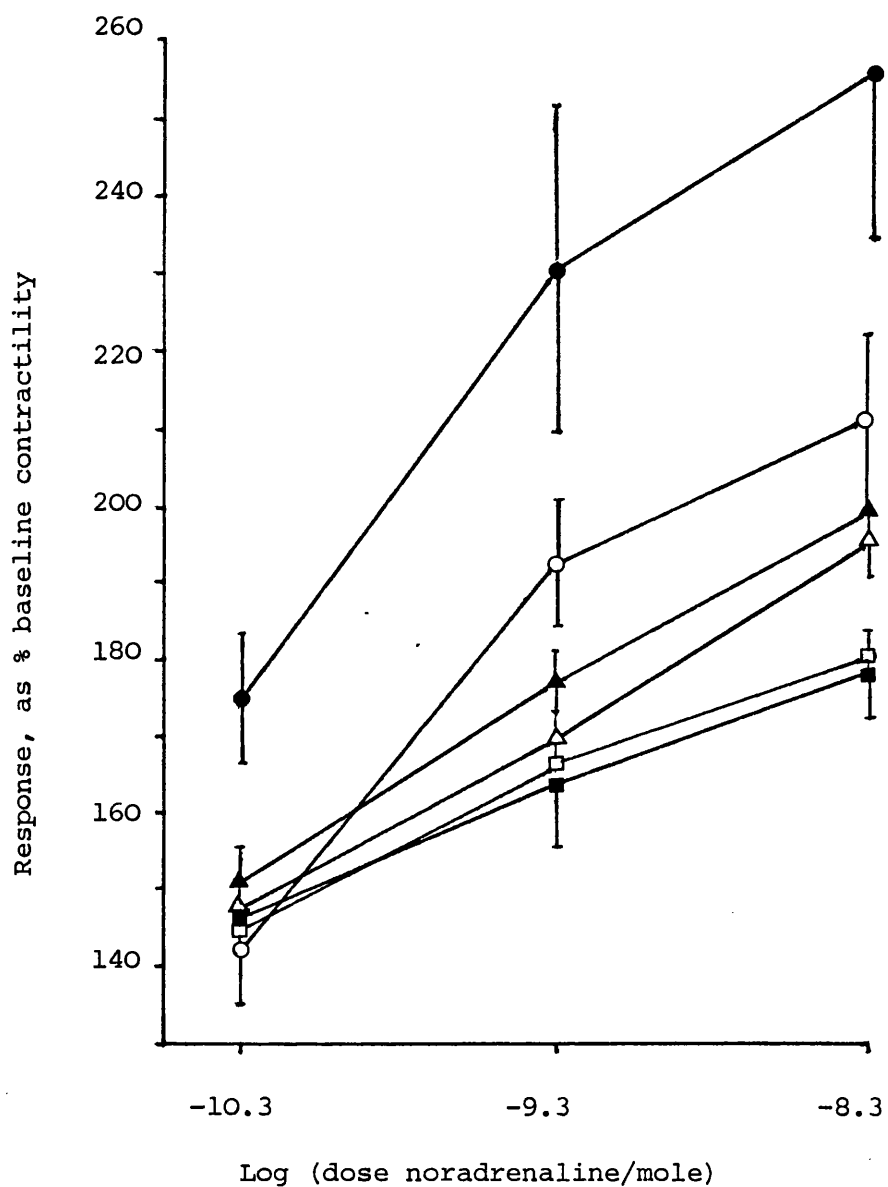
Table 3.6(vi)

The effects of 4 weeks of daily caffeine administration on baseline contractility (g) of the isolated perfused hearts (under 2g basal tension) from groups of rats and the effects of three doses of noradrenaline (added 'in vitro') on this contractility. All values expressed as mean \pm s.e.m. for the group.

<u>Group</u>	<u>Baseline</u> <u>contractility</u>	<u>Response to dose of noradrenaline (% baseline</u> <u>contractility)</u>		
		<u>5×10^{-11} mole</u>	<u>5×10^{-10} mole</u>	<u>5×10^{-9} mole</u>
L (n=9)	6.56 \pm 1.23	142.57 \pm 6.85	192.43 \pm 8.19	211.50 \pm 10.87
D (n=9)	6.17 \pm 0.59	175.59 \pm 8.46	231.09 \pm 20.96	255.36 \pm 21.13
LC25 (n=11)	8.61 \pm 0.58	147.33 \pm 4.72	169.63 \pm 3.70	195.43 \pm 5.27
DC25 (n=12)	8.06 \pm 0.30	151.09 \pm 4.22	177.68 \pm 4.39	198.90 \pm 6.96
LC50 (n=11)	9.34 \pm 0.49	144.67 \pm 3.26	166.64 \pm 4.45	179.79 \pm 3.61
DC50 (n=9)	8.33 \pm 0.71	146.78 \pm 6.99	164.20 \pm 8.39	179.14 \pm 6.21

For key to abbreviations for groups see Table 3.6(v)

Fig. 29 The effect of noradrenaline upon contractility of isolated perfused hearts from lean and dietary obese rats and the changes induced by chronic treatment (4 weeks) with two doses of caffeine. All values shown as mean \pm s.e.m.



Key

- (L), lean, 4 weeks untreated (n=9)
- (D), dietary obese, 4 weeks untreated (n=9)
- △—△ (LC25), lean, caffeine 25mg/kg (n=11)
- ▲—▲ (DC25), dietary obese, caffeine 25mg/kg (n=12)
- (LC50), lean, caffeine 50mg/kg (n=11)
- (DC50), dietary obese, caffeine 50mg/kg (n=9)

Table 3.6(vii)

The number of arrhythmias (missed beats and ectopic beats) occurring in isolated perfused hearts from groups of rats after each of three doses of noradrenaline (during first 3 minutes after each dose).

Values shown for each preparation.

<u>Group</u>	<u>Dose of noradrenaline</u>		
	<u>5×10^{-11} mole</u>	<u>5×10^{-10} mole</u>	<u>5×10^{-9} mole</u>
L	1,1,1,0,36,0,	0,2,0,1,0,9,	2,0,0,1,2,16,
(n=9)	1,0,0	2,0,0	2,0,0
D	0,0,0,0,0,0,	1,1,2,0,3,1,	143,2,1,0,2,2,
(n=9)	0,0,0	22,0,0	0,0,0
LC25	1,2,0,0,0,0,	3,21,0,0,0,1,	1,1,0,0,5,3,
(n=11)	0,0,0,1,1	3,10,0,5,1	0,0,12,106,1
DC25	0,0,0,0,0,0,	0,2,0,1,1,0,	0,1,0,6,0,0,
(n=12)	0,0,0,0,0,0	0,9,11,0,0,3	2,0,0,15,3,1
LC50	0,0,7,0,23,3,	0,2,2,0,3,2,	0,8,55,2,2,0,
(n=11)	0,1,2,0,1	0,1,0,2,4	2,1,0,0,3
DC50	0,0,0,0,0,0,	0,5,0,2,2,0,	1,3,2,36,10,4,
(n=9)	0,0,0	4,8,0	7,2,0

No significant differences in the number of arrhythmias were found between the groups, with any dose of noradrenaline (using the Mann-Whitney U-test). For key to abbreviations for groups see Table 3.6(v).

d) The responses to doses of acetylcholine

Considering first the duration of cardiac arrest induced by 5×10^{-9} mole acetylcholine (see Table 3.6(viii)a)): the hearts from the dietary obese rats (D) stopped for longer than hearts from lean controls (L), (3.87 ± 0.83 and 2.30 ± 0.73 seconds, respectively) $p < 0.05$. The hearts from both lean caffeine-treated groups (LC25 and LC50) also stopped for longer than those from L (both $p < 0.001$). The hearts from both caffeine-treated dietary obese groups (DC25 and DC50) also stopped for longer than those from D ($p < 0.01$ for DC25 vs D and $p < 0.001$ for DC50 vs D). There was no difference between the duration of cardiac arrest of hearts from the two lean caffeine treated groups, LC25 and LC50, however hearts from DC50 stopped for longer than those from DC25. Thus hearts from obese animals chronically pre-treated with the two doses of caffeine showed a dose-dependent lengthening of cardiac arrest periods induced by 5×10^{-9} mole of acetylcholine. However in hearts from the lean animals both doses of caffeine produced a similar lengthening of the cardiac arrest period induced by 5×10^{-9} mole acetylcholine. Secondly, considering the duration of cardiac arrest induced by 5×10^{-8} mole of acetylcholine (again, see Table 3.6(viii)a)). There were no differences between data obtained from untreated lean and obese rats. Again, hearts from both dietary obese groups which had received caffeine, had longer cardiac arrest periods than hearts from dietary obese controls ($p < 0.001$), furthermore the high dose of caffeine was associated with an additional lengthening of the cardiac arrest period. Therefore, again administration of caffeine resulted in a dose-dependent lengthening of the cardiac arrest period induced by acetylcholine. Caffeine treatment did not affect the duration of

Table 3.6(viii)

Responses of isolated perfused hearts from groups of rats to two doses of acetylcholine. All values shown as mean \pm s.e.m. for group.

a) Duration of cardiac arrest (seconds)

<u>Group</u>	<u>Dose of acetylcholine</u>	
	<u>5×10^{-9} mole</u>	<u>5×10^{-8} mole</u>
L (n=9)	2.30 \pm 0.73	8.16 \pm 1.16
D (n=9)	3.87 \pm 0.83	7.78 \pm 0.82
LC25 (n=11)	4.69 \pm 1.04	8.95 \pm 1.09
DC25 (n=12)	6.11 \pm 0.99	13.78 \pm 1.44
LC50 (n=11)	4.41 \pm 0.46	10.13 \pm 1.37
DC50 (n=9)	10.18 \pm 1.51	16.69 \pm 1.89

b) Length of time to recover regular beat (seconds)

<u>Group</u>	<u>Dose of acetylcholine</u>	
	<u>5×10^{-9} mole</u>	<u>5×10^{-8} mole</u>
L (n=9)	12.30 \pm 2.65	38.47 \pm 2.97
D (n=9)	11.61 \pm 2.67	31.20 \pm 2.47
LC25 (n=11)	14.08 \pm 2.32	40.68 \pm 3.70
DC25 (n=12)	19.05 \pm 3.20	46.29 \pm 4.01
LC50 (n=11)	18.75 \pm 1.08	39.99 \pm 1.06
DC50 (n=9)	18.23 \pm 1.63	37.31 \pm 2.00

For key to abbreviations for groups see Table 3.6(v)

cardiac arrest induced by acetylcholine in hearts from lean rats.

Considering the time required to resume regular beating rate after 5×10^{-9} mole acetylcholine (see Table 3.6(viii)b)), there was no significant difference in the response of hearts from lean (L) and dietary obese (D) groups. However, both doses of caffeine increased the recovery time for hearts from obese rats (DC25 and DC50), both $p < 0.001$, although this effect did not appear to be dose-dependent. A similar trend was seen in the hearts from the caffeine-treated lean rats. Hearts from LC50 had significantly longer recovery times than hearts from L ($p < 0.001$) and LC25 ($p < 0.01$), but hearts from LC25 did not have significantly longer recovery times than hearts from L. Lastly, considering the recovery times after 5×10^{-8} mole acetylcholine (again, see Table 3.6(viii)b)): hearts from obese rats (D) recovered more quickly than hearts from lean rats (L), $p < 0.01$. Chronic administration of caffeine (LC25 and LC50) did not alter the recovery time seen in hearts from lean rats (L). However, pre-treatment with 25mg/kg caffeine (DC25) in obese rats resulted in an increased recovery time compared with untreated obese rats (D), $p < 0.001$. This lengthened recovery time was also seen with the higher dose of caffeine in obese rats (DC50), although it was less significant at $p < 0.05$ (the value for LC50 of 37.31 ± 2.00 being similar to that seen for lean controls, 38.47 ± 2.97). Thus, while the effect of caffeine on the response of the isolated hearts to acetylcholine is to lengthen the duration of cardiac arrest, its effects on recovery time are less clear. Similarly contradictory findings are seen with the effect of dietary obesity. There was a small but significant increase in the duration of cardiac arrest induced by the lower dose of acetylcholine ($p < 0.05$), which was not

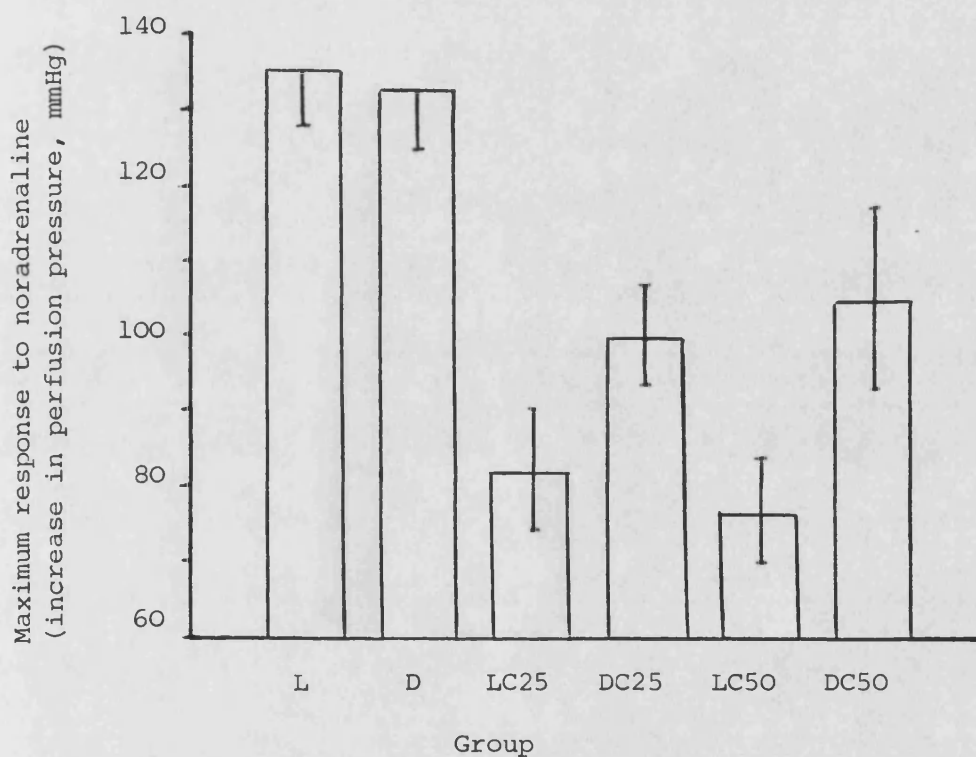
seen with the higher dose, while the time for recovery following each dose of acetylcholine was reduced (significantly for the higher dose of acetylcholine, $p < 0.01$).

3.6.6 'In vitro' studies: the isolated perfused mesenteric vasculature

The maximum response (expressed as maximum increase in perfusion pressure, mmHg) induced by bolus injections of noradrenaline in the isolated perfused mesenteric bed were recorded for all groups. The results were as follows: L, 135.30 ± 7.60 mmHg; D, 133.00 ± 8.50 mmHg; LC25, 82.22 ± 8.17 mmHg; DC25, 99.82 ± 6.80 mmHg; LC50, 76.41 ± 6.80 mmHg; DC50, 104.98 ± 11.91 mmHg. These results are represented graphically in Fig.30. There was no significant difference between the values for L and D. Caffeine pre-treatment, at both dose levels, resulted in a significant reduction in the maximal response. Tissues from LC25 and LC50 both gave significantly lower maximal responses than tissues from L (both $p < 0.001$). Tissues from DC25 gave significantly lower maximal responses than tissues from D ($p < 0.001$) and those from DC50 also gave significantly lower maximal responses than tissues from D ($p < 0.01$). There were no significant differences between maximal responses of tissues from DC25 and DC50 or between tissues from LC25 and LC50. However tissues from DC50 gave greater maximal responses than tissues from LC50 ($p < 0.001$) and tissues from DC25 gave significantly greater maximal responses than tissues from LC25 ($p < 0.01$). Thus, the presence of dietary obesity appeared to attenuate the reduction in maximal response which occurred with caffeine treatment.

Fig.30 Change in the maximum response induced by noradrenaline in the isolated perfused mesenteric vasculature from lean and dietary obese rats by chronic (4 weeks) treatment with two doses of caffeine.

All values mean \pm s.e.m.



Key

L = Lean, 4 weeks untreated (n=6)

D = Dietary obese, 4 weeks untreated (n=6)

LC25 = Lean, caffeine 25mg/kg (n=11)

DC25 = Dietary obese, caffeine 25mg/kg (n=12)

LC50 = Lean, caffeine 50mg/kg (n=12)

DC50 = Dietary obese, caffeine 50mg/kg (n=11)

Chapter 4 : DISCUSSION

CHAPTER 4 : DISCUSSION

4.1 THE USE OF THE DIETARY OBESE RAT MODEL FOR OBESITY.

Many animal models of obesity have been developed including neural models such as rats with ventromedial hypothalamic lesions; endocrine models such as ovariectomised animals; genetic models including the ob/ob mouse and the fa/fa Zucker rat and finally dietary obese models (see section 1.2.5). A number of factors were considered when selecting the most suitable animal model for these studies. The first requirement was that the obesity should be induced by similar mechanisms to those commonly associated with the human disease. As no neural dysfunction has been identified in most forms of human obesity the lesioned animal models were not considered appropriate. Secondly, although some endocrine imbalance may occur as a result of the establishment of obesity (see section 1.2.3c), there is little evidence to suggest that endocrine disorders are a major cause of the condition in man. Thirdly, the genetically obese animal models were excluded because of the occurrence of gross metabolic alterations not mirrored in the human disease. For example, the fa/fa rat displays hyperlipidaemia and the ob/ob mouse hyperglycaemia and hyperinsulinaemia (see section 1.2.5). The dietary obese rat was selected as the most suitable model for human obesity because the animals appear to become fat due to overeating and lack of physical exercise. The three most common methods of producing dietary obesity in rodents are provision of a high fat diet, a high sugar diet or a varied palatable diet, often referred to as a "supermarket" or "cafeteria" diet (Sclafani, 1984). The varied palatable diet was chosen because the food items used (Rice Krispies, digestive

biscuits, luncheon meat and chocolate) allow for a more balanced intake of nutrients than is possible with either the high fat or high sugar diets. Earlier studies have shown that this diet produced a greater rate of weight gain than standard laboratory chow (Pleece, 1983 ; Agadah, 1985). The obesity produced by this model is moderate compared to that occurring with the neural and genetic models.

However, the weight gain seen with the palatable diet was unpredictable, with some rats gaining rapidly and continuing to gain weight over many weeks, while others only showed a moderate gain in weight over that of chow-fed controls. As the rats were housed in groups of six it was not possible to measure the food and water intake of each individual rat. Therefore, one could not determine whether the rats who showed little weight gain did so because they ate less than their fatter counterparts or whether they were more active and/or had a higher metabolic rate. Single housing would have been required to investigate this more fully. However, single housing itself alters food intake (Agadah, 1985), thus following the poor responders by housing them individually would not have given an accurate indication of their behaviour in a group.

In addition to the variation in weight gain seen within groups, variation was also apparent for the mean weight gain of different groups receiving the palatable diet. It can be clearly seen in Table 3.2(v) that the mean weight gain per rat over the first 4 weeks of receiving the palatable diet varied from 48g (for MAZ) to 77g (for D). The weight gain for the age-matched chow-fed controls (L) in that particular experiment was 36g and so although all the groups of animals offered the palatable diet gained significantly more weight than chow-fed controls (and may therefore be considered 'dietary

obese') the weight gain in one group was almost 30g greater (mean per rat) than in others.

One possible explanation for some of this variation in the rate of weight gain is the effect of seasonal variation on lipid metabolism. Although experimental conditions were standardised as far as practical, it was not possible to study all groups together and the starting dates for the groups had to be staggered over at least 6 to 8 weeks to allow sufficient time for all the monitoring procedures to be carried out. Indeed, the data shown in Table 3.2(v) was collected over more than 12 months. Heaf et al (1979) showed that the effect of food intake on plasma free fatty acid levels in rats ranged from a lowering of levels in winter to an increase in summer. No seasonal variation was observed in the corresponding blood glucose values however. Heaf's study found that both fasting and postprandial plasma free fatty acid levels varied widely, with minima occurring in late January and May. A similar dicyclic variation was observed by Barrett (1964), with minima occurring in June and December. In the latter study the peak values, occurring February/April and August/September, were approximately 30% higher than the low figures, the difference being highly significant ($p < 0.001$). The dicyclic seasonal variation in plasma free fatty acids corresponds to a similar pattern seen in serum cholesterol levels (Thorp & Waring, 1962; Edgren, 1963).

Increases in plasma free fatty acids have been observed in situations of stress and/or arousal due to stimulation of sympathetic nervous system activity. Heaf et al (1979) suggested that the increased arousal of experimental animals in the summer months was related to the seasonal cycles of aggression associated with the

dispersal of rodent populations in the wild (Krebs & Myers, 1974) and that the increased levels of free fatty acids induced by feeding in the summer reflected an enhanced sympathetic activity as a result of the greater state of arousal. Similar seasonal variations in adrenocortical and thyroid function have been shown to occur (Thorp, 1963). An external synchroniser of the observed seasonal effects would appear to be necessary, however, no such synchroniser has been identified. In some studies the powerful effect of photoperiod has seemed a likely candidate. However, in Heaf's study and also in the dietary obese rat studies described here, the variation in photoperiod was eliminated by the use of a constant fixed light:dark ratio. One possible synchroniser or inducer of metabolic rhythms which was not fully controlled in Heaf's study was the laboratory temperature. The laboratory was maintained at 21°C during the winter but in the summer the temperature might have risen if temperatures outside rose above 21°C as there were no facilities to prevent an increase in temperature. (The same limitation was not true for the studies of dietary obese rats described here, however, as the animal housing rooms were maintained at 22°C during the whole year). However, change in temperature seems an unlikely explanation for the variations in lipid metabolism in view of the finding by Jouanneteau et al (1975) that the effect of temperature on a number of measurements of lipid metabolism in the rat was itself seasonally dependent. Again in experiments with standardised lighting and temperature, seasonal variations have been detected in the glycogen content of diaphragm muscle (Pessacq & Gagliardino, 1975) and in blood lipid and a number of other blood measurements in two different strains of mice (Cuendat et al, 1975). Since susceptibility to

seasonal influences may be a characteristic of lipid metabolism in the rat, it is interesting that a number of metabolic effects have been detected in adipose tissue (Boright et al, 1962; Blackard & Cameron, 1967).

The seasonal variations observed in lipid metabolism and sympathetic nervous system activity may affect energy expenditure and fat deposition (and hence weight gain) in dietary obese and lean rats. As stated earlier, the data for the weight changes shown in Table 3.2(v), for the lean, obese and weight-reduced rats was obtained over a period of more than 12 months. Each group of rats was monitored for a period of 8 weeks (see section 2.8.6 for experimental details) and the monitoring periods were as follows: for the lean, untreated group (L) April-June 1986; for the dietary obese, untreated group (D), April-June 1986; for the dietary obese, control-dosed group (CD), Oct-Dec 1986; for group WD (palatable diet withdrawn), Oct-Dec 1986; for the amphetamine-treated group (AMPH), May-July 1987; for the fenfluramine-treated group (FEN), June-July 1987; and for the mazindol-treated group (MAZ), June-Aug 1987. It is also of interest to note that the anorectic drugs mazindol, fenfluramine and amphetamine all have the effect of increasing plasma free fatty acid concentrations (see section 1.4) and so seasonal variations in lipid metabolism may possibly affect the actions of these drugs.

Rietveld et al (1980) found a levelling off of the mean growth curve of rats during February and March, for which no systematic correlation between food intake and increase in body weight could be demonstrated. They also found seasonal fluctuations in food intake in rats with a decrease occurring during midsummer (August) and maximal intake in January and February. Results of a study by Ikononov and

Stoynev (1982) also found distinct seasonal variations in food intake in rats, values being maximal in autumn and winter and minimal in spring and summer. Thus, seasonal alterations in food intake may also have contributed to the wide range of mean weight gain seen for groups of dietary obese rats after similar time periods of receiving the varied palatable diet. Comprehensive monitoring of food intake of the dietary obese rats throughout the year would be required to clarify this matter.

4.2 THE CHANGES OCCURRING IN BODY WEIGHT, BLOOD PRESSURE AND HEART RATE WITH TIME IN LEAN AND DIETARY OBESE RATS.

Unless otherwise stated, the time periods given refer to the number of weeks from the start of the study during which the group had received laboratory chow with or without the palatable diet.

It has been clearly demonstrated that the dietary obese rats exhibited elevated blood pressures compared with age-matched lean (chow-fed) controls (see Table 3.1(iv) and Fig.8). Several of the possible mechanisms of obesity-associated hypertension are discussed below.

One mechanism is that overfeeding causes an increase in serum triiodothyronine (T_3) (Danforth et al, 1979). Williams et al (1977) found, in the rat, that T_3 increased β -adrenergic receptor number and thus could increase the sensitivity to pressor mechanisms. This has been suggested as a contributor to the hypertension which may be seen in some overweight patients. The net availability of carbohydrate appears to be a critical factor in this response since isocaloric reduction of carbohydrate reduces serum T_3 (Danforth et al, 1979). This mechanism assumes that the obese hypertensive overeats or at

least consumes relatively large quantities of carbohydrate. The dietary obese rats in the studies described here were indeed hyperphagic and consumed more energy and carbohydrate than the lean chow-fed controls. Therefore, this proposed mechanism may partially explain the increase in blood pressure observed. Measurements of plasma T_3 in obese rats and in lean controls would be of interest in future work, to further investigate this possibility.

The effect of dietary intake on sympathetic neuronal activity provides another possible mechanism for the hypertension associated with obesity. Studies of the activity of the sympathetic nervous system in human obesity have generally shown only very small differences between lean and obese individuals. James et al (1981) found small increases in sympathetic function in obese women compared to lean, however these differences were not significant. In a study of healthy male subjects (Peterson et al, 1988), depressions of both sympathetic and parasympathetic activity were found to have a weak, but significant correlation with increasing percentages of body fat. These apparently contradictory findings may be the result of sex differences in autonomic function in obesity, which may in turn be due to the different body fat distribution patterns seen in men and women (predominantly upper body segment fat in males and lower body segment fat in females). However, although weight and percentage of body fat 'per se' may not always correlate significantly with sympathetic activity, alterations in dietary intake, which can produce weight gain or loss, appear to have much more consistent effects. The effect of dietary carbohydrate, in particular, on sympathetic neuronal activity has been the focus of several studies of obesity-related hypertension. Utilising techniques to measure the

turnover rate of noradrenaline in the heart, fasting has been shown to suppress and overfeeding with sucrose to stimulate the sympathetic nervous system in rats (Landsberg & Young, 1981a). Studies in the spontaneously hypertensive rat have also demonstrated that fasting or restriction of energy intake lowers blood pressure and that overfeeding with sucrose, but not with fat, increases blood pressure (Landsberg & Young, 1981a). This latter finding was contrary to the results of several other studies in normotensive animals and in man, which have shown increases in blood pressure with a high fat diet (see section 1.5.7a)). Obese patients placed on a reduced carbohydrate intake showed a fall in metabolic rate, a reduction in the cardiovascular indices of sympathetic activity and a fall in venous noradrenaline concentrations (James et al, 1981). These changes were not so evident in those obese patients who were also hypertensive, which suggests that there may be an alteration in the control of catecholamine metabolism in obese hypertensive patients.

Enhanced sympathetic nervous system activity has been reported in animals fed a varied palatable diet (Young et al, 1982), while in most other animal models of obesity, such as genetically obese strains and lesioned animal models of obesity, reduced sympathetic activity has been found (Bray, 1987c). This difference may occur because the dietary obese rats start with a normal metabolism and become obese mainly as a result of hyperphagia. There is usually a marked increase in both total energy and carbohydrate intake in these rats. However, genetically obese, and some lesioned, animals have metabolic disturbances before the onset of obesity, which may be reflected in alterations in sympathetic neuronal activity. Thus the hyperphagia and increased carbohydrate intake of the dietary obese

rats in the studies described here may be partly responsible for the simultaneous elevation of heart rate and blood pressure seen. Further evidence to support this theory includes the fact that although body weight continued to increase steadily in the dietary obese rats up to the end of the 20 week study period, both blood pressure and heart rate peaked earlier in the study (after 6 to 10 weeks for blood pressure and after 10 weeks for heart rate) and then showed a small decline between 10 and 20 weeks. Both total energy intake and carbohydrate intake fell by approximately 25% after 8 weeks and this lower intake was maintained to the end of the 20 weeks of the study. The reduction in food intake occurred at approximately the same stage in the study as the decline in heart rate and blood pressure.

The weak correlation between weight and blood pressure seen within the dietary obese group alone, and the stronger correlation seen with both lean and obese groups combined, could also be explained, at least in part, by this theory. For although some of the dietary obese rats gained weight only a little faster than the lean controls, they may have been consuming equally large quantities of carbohydrate as the fatter members of the group, but due to greater energy expenditure (caused possibly by increased physical activity and/or an increased metabolic rate) this may have resulted in less weight gain. If the sympathetic activity were affected by food intake, rather than by body weight 'per se' (as discussed earlier in this section) then the leaner dietary obese rats would exhibit a similar increase in sympathetic activity as the fatter members of the group (and thus be expected to have similar increases in heart rate and blood pressure). This would, of course, result in a poor correlation of weight and blood pressure within the group. However,

if one assumes that all the rats in the dietary obese group ate larger quantities of energy and carbohydrate than the chow-fed group, and as a result of this were also heavier (though with a wide range of weights within the groups) an enhancement of sympathetic activity (as reflected in increased heart rate and blood pressure) would be expected in the dietary obese group as a whole compared with the lean controls. This would result in a greater correlation between weight and blood pressure for the two groups combined, as was seen in the experiments described here.

Elevated cardiac output has been found in the obese (Alexander, 1963; Messerli et al, 1981). However, after correction of cardiac output values for surface area, the differences between lean and obese were no longer apparent. Therefore, the contribution, if any, of increased cardiac output to the hypertension associated with obesity is uncertain.

A hypothesis linking diet, obesity, body fat distribution, insulin and hypertension was proposed by Landsberg (1986). He observed that not all obese persons were hypertensive and that cardiovascular disease in general and hypertension in particular, are associated with fat accumulation in the abdomen and chest (android or upper body obesity) rather than in the gluteal and femoral regions (gynoid or lower body obesity) (Lapidus et al, 1984; Larsson et al, 1984). Insulin levels, both basal and in response to a glucose challenge, were higher in obese subjects with upper body obesity, as compared with those with lower body obesity (Kissebah et al, 1982; Krotkiewski et al, 1983). Thus, both hyperinsulinaemia and hypertension segregate together in the group of obese subjects characterised anthropometrically by an upper body fat distribution.

Other recent studies show a close correlation between hypertension and hyperinsulinaemia in the same obese subjects (Modan et al, 1985; Manicardi et al, 1986). In both animals and man, moreover, evidence from physiological studies suggests that insulin predisposes to hypertension by stimulating sodium reabsorption and by stimulation of the sympathetic nervous system. By altering the capacity of the kidney to excrete sodium, insulin would be expected to change the 'pressure-natriuresis' relationship so that higher renal (and hence arterial) perfusion pressures would be necessary to excrete the same amount of salt (Kolanowski, 1981). The hyperinsulinaemia of obesity may, therefore, be one important factor contributing to the 'natriuretic handicap' displayed by hypertensive patients; the inhibitory effect of insulin on sodium excretion would mean that sodium balance could be maintained only by an increase in blood pressure, once the recruitment of other natriuretic mechanisms was exhausted. Insulin has also been demonstrated to increase sympathetic nervous system activity (Landsberg & Young, 1985). The hyperinsulinaemia of obesity, therefore, may stimulate the sympathetic nervous system excessively. The noradrenaline released from the nerve terminals in the kidney, heart and blood vessels would raise blood pressure by enhancing sodium reabsorption, increasing cardiac output and increasing peripheral resistance (Landsberg & Young 1981b). The antinatriuretic effects of both catecholamines and insulin would prevent the kidney from compensating for the elevated blood pressure consequent to sympathetic stimulation of the heart and vasculature. The actions of insulin on the kidney and sympathetic nervous system, therefore, provide a potential explanation for the epidemiological association of hypertension and hyperinsulinaemia

seen in obese subjects.

Further investigations in our own dietary obese rat model for obesity, which would be of interest, include the determination of plasma insulin levels and also plasma catecholamine levels (as mentioned earlier). The question of body fat distribution in the rat has not been considered in any of the published studies of dietary obesity in this animal. Whether there are equivalent 'gynoid' and 'android' body fat distribution types in rats and whether or not such subtypes would have any metabolic significance, have yet to be determined.

The role of salt intake in the hypertension associated with obesity has been the subject of some controversy. Dahl (1972) gave a simple and direct explanation for the obesity-blood pressure correlation. He felt that the obese ate more than the lean, therefore they consumed more salt, and the proximate cause of the increased blood pressure was increased salt intake. A report by Watson and Langford (1982) gave partial support to this theory. Weight and urinary excretion of a number of electrolytes was found to be correlated. However, when data was subjected to stepwise regression, a weak, but significant, effect of sodium remained. Hiramatsu et al (1981) proposed that with a high salt intake, as a result of intake of large quantities of food by the obese, aldosterone/plasma renin activity would be abnormally elevated and would contribute to retention of salt and water, producing hypertension through expanded extracellular fluid volume. However, some recent studies have failed to support this hypothesis. Mujais et al (1982) showed that expressing blood volume in relation to body surface area eliminated the differences between obese and non-obese subjects. Messerli et al

(1981) found that the predicted total blood volume was similar in normal, mildly obese and obese patients; the ratio between measured and predicted blood volume was almost equal in the three groups, except in the lean patients with established hypertension in whom volume contraction was seen. A study by Reisin and Frohlich (1982) suggested that obesity raised blood pressure directly, or at least that weight loss directly lowered blood pressure even if salt intake was held constant. Tuck et al (1981) arrived at a similar conclusion in a study where the very obese were put on a very low calorie intake and one of two levels of sodium intake. The fall in blood pressure was proportional to the weight loss and the blood pressure fall by the end of the study period was the same for both diets, although one contained twice as much salt as the other. However, both diets contained less sodium than the diets the patients were eating before the study.

There is a considerable body of evidence to support the role of a high sodium intake in the genesis and maintenance of hypertension in a 'salt-sensitive' subgroup of the population (see section 1.5), even if, as Reisin's study (1982) indicates, sodium restriction is not essential for the hypotensive effect of weight reduction. The dietary obese rats used in the studies described here had greater sodium intakes than chow-fed controls. This may have been a contributory factor in the elevated blood pressures seen in these rats.

In summary, the elevation of blood pressure seen in the dietary obese rats in the studies described here may have been associated with a number of factors. Firstly, it is likely that the increased weight 'per se' was involved in the elevation in blood pressure, in

view of the vast array of literature in which weight and blood pressure have been shown to be strongly associated. The possible mechanisms have not been conclusively established. However, it may be that the hyperinsulinaemia often found in obesity may be one factor involved. Determination of plasma insulin levels in the dietary obese rats would be necessary to discover whether or not hyperinsulinaemia was involved in the elevation in blood pressure seen. The greater intake of energy and carbohydrate by the dietary obese rats might also have played a role in the elevation of both blood pressure and heart rate seen by induction of an increase in sympathetic nervous system activity. Further studies involving the determination of plasma levels of triiodothyronine (T_3) and noradrenaline would be necessary to detect any alterations in sympathetic activity in the dietary obese rats and to determine whether elevated T_3 levels were involved in these alterations. Urinary levels of the major metabolites of noradrenaline would also provide valuable information with respect to sympathetic activity. The higher level of sodium intake in the dietary obese rats might also be a contributory factor in the elevation in blood pressure seen.

4.3 PLASMA SODIUM AND POTASSIUM LEVELS TOGETHER WITH THE INTAKE OF DIETARY SODIUM, POTASSIUM, CALCIUM AND VARIOUS NUTRIENTS IN LEAN (CHOW-FED) AND DIETARY OBESE RATS.

Analysis of the nutrient intake of lean (chow-fed) and dietary obese (chow plus palatable diet) rats revealed that the sodium intake of the dietary obese rats was between 350 and 500% that of the lean controls (see section 3.1.1). The intake of potassium in the dietary obese rats, however, was approximately half that of the lean

controls. Evidence for the hypertensive effect of dietary sodium and for the protective or hypotensive effect of dietary potassium was discussed earlier in section 1.5. Thus, from the balance of the two electrolytes in the diet of the rats consuming the palatable foods, it would appear that these animals might be predisposed to elevation in blood pressure. An earlier study, using this particular palatable diet (Kirby, 1984), showed that supplementing the salt intake of the chow-fed rats, to give a similar level to that of the dietary obese rats (by giving 1% saline in place of drinking water), produced an initial rise in blood pressure during the first one to two weeks of the study, comparable to that seen in the dietary obese group. The blood pressure then fell to approximately the same level as the lean controls (given plain water). It was suggested that the initial rise in blood pressure seen in the dietary obese rats was due to the increase in salt, but that the sustained rise was due to the developing obesity in these animals. In the light of the low potassium intake, it seems that potassium may also play a role in the elevation in blood pressure seen in the dietary obese rats. Another method of distinguishing the weight effect from the salt effect in these dietary obese rats would be to provide a 'low salt' palatable diet. Ideally, such a diet would provide similar proportions of nutrients to those of the normal palatable diet (see Appendix 1) in all respects except for the sodium content. Such a diet could be as follows: firstly replacing the Rice Krispies (1110mg sodium per 100g) with Puffed Wheat (4mg per 100g); the digestive biscuits (440mg per 100g) with cream wafer biscuits (70mg per 100g); the chocolate (118mg per 100g) would remain unchanged and the luncheon meat (1050mg per 100g) would be replaced with egg cooked with lard and water (140mg

per 100g, value for whole egg). The proportions of other nutrients in the diet apart from sodium would be virtually unchanged (including potassium and calcium). A pilot study would, of course, be required to establish that the rats found the diet palatable enough to overeat and become obese.

Plasma sodium concentrations were determined at 1, 2, 3, 4, 8, and 20 weeks in lean and dietary obese and also in a group of rats which had received the palatable diet for 4 weeks before having it withdrawn for 4 weeks (see section 3.4 for results). Standard values for the concentrations of sodium and potassium in the rat are 135mM for Na^+ and 4.9mM for K^+ (Waynforth, 1980). The values for the rats used in these studies were a little higher than average, though not abnormally so. The plasma sodium concentrations were higher in the dietary obese rats than lean rats after 2 weeks, then the values fell to lower than lean for the next 2 weeks, possibly as the result of a compensatory mechanism. It is of interest to note that the rise in blood pressure observed in the lean rats given 1% saline in place of drinking water in the study by Kirby (1984), occurred after one week of treatment but by two weeks it had fallen back to a similar level to that seen in the lean non-salt-supplemented rats. It is possible that a similar compensatory mechanism was also responsible for this phenomenon. After 8 weeks the plasma sodium levels were similar for lean and obese groups, but by 20 weeks, the value for the dietary obese rats was again higher than for lean. Thus, it may be that any compensatory mechanisms can be overcome with prolonged intake of large quantities of sodium, or that the developing obesity may introduce another factor which the compensatory mechanisms can not overcome. When the mean plasma sodium concentration of the 'diet

withdrawn' rats was compared with the values for lean and obese rats at 8 weeks there were no significant differences between the three values.

Considering the plasma potassium concentrations: these values were similar in the lean and obese rats at 4 and 8 weeks, while at 20 weeks the mean value for the obese rats was higher than for the lean. This is surprising when one considers that the potassium intake of the dietary obese rats was only approximately 50% of that of the lean rats.

It was noted that blood samples from the dietary obese rats were more susceptible to haemolysis than those from the lean animals. Although all the samples were treated in an identical manner (see section 2.3), the plasma samples obtained from the dietary obese rats were often orange-red in colour (normal samples being straw-coloured). If haemolysis had occurred, the readings obtained for potassium (K^+) concentration would be falsely elevated due to the leaking out of potassium from the erythrocytes, as the intracellular potassium concentration is higher than that in the extracellular fluid (Davis et al, 1985). Thus, values for potassium concentration of haemolysed samples were excluded from any calculations. Altered properties of the erythrocyte membrane in obese children have been reported by Ciancarelli-Tozzi et al (1987). An investigation of the properties of the erythrocytes from lean and obese rats might be of interest in further studies. Measurements of the urinary excretion of sodium and potassium might also yield useful information about the handling of these electrolytes in lean and dietary obese rats.

The calcium intake of the dietary obese rats was found to be only 20-30% that of the lean chow-fed controls (see section 3.1.1). A

relative or absolute deficiency of calcium has been shown to be present in some cases of human hypertension (see section 1.5.6b)), although, as with sodium, there seems to be considerable heterogeneity in the blood pressure response to calcium. However, it is possible that the low calcium content of the diet may have contributed to the elevation in blood pressure seen in the dietary obese rats. In future studies, it would be interesting to monitor the concentrations of calcium in the plasma and urine from lean and dietary obese rats.

The intake of fat by the dietary obese rats ranged from approximately 400-670% that of the lean chow-fed controls. The laboratory chow pellets had a very low fat content of only 2.4% and therefore although the most preferred item of the palatable diet only had a fat content of 2%, the contribution of fat from the other items (digestive biscuits, 20.5% fat; luncheon meat 26.9% fat and chocolate, 29.3% fat) resulted in a considerable increase in total fat intake. A fat-enriched diet has been shown to cause increases in blood pressure in rabbits (Burstyn & Husbands, 1980) and a reduction in saturated fat intake has been shown to reduce blood pressure in man, with a rebound increase in blood pressure occurring when the saturated fat intake was restored to pre-intervention values (Iacono, Dougherty & Puska, 1986). In addition, a study by Smith-Barbara et al (1980) showed that the elevation in blood pressure in Sprague-Dawley rats, caused by a high-salt diet, was most marked when the diet was also high in saturated fat content (see section 1.5.7a) for a fuller discussion of the role of fat in hypertension). As saturated fatty acids accounted for approximately 50% of the fatty acid content of the palatable food items (see Appendix 2), this elevation in

saturated fat intake may also have contributed to the higher blood pressure levels seen in the dietary obese rats.

The dietary fibre intake of the dietary obese rats was slightly higher than that of the lean, chow-fed controls (116-181%). There is evidence that a high fibre diet may offer protection against the hypertensive effect of a high fat diet (see section 1.5.7a), though it is not possible to determine if this was the case in this dietary obese animal model.

The carbohydrate intake of the dietary obese rats was also slightly higher than that of the lean controls. A high intake of carbohydrate has been shown to produce an increase in blood pressure in man (Affarah et al, 1986) and therefore, this factor may have been contributory to the elevation in blood pressure seen in the dietary obese rats. The protein intake of the dietary obese rats was lower than that of the lean controls (approximately 60% lean value), though it is unclear what effects, if any, this might have on cardiovascular performance.

Water intake was always greater in the dietary obese rats than in the lean, probably partly as a result of the greater salt intake in the dietary obese animals.

4.4 INTERPRETATION OF THE CHANGES IN 'IN VITRO' RESPONSES OF CARDIOVASCULAR TISSUES FROM LEAN AND DIETARY OBESE RATS.

The isolated perfused heart and mesenteric vascular bed preparations were used for the majority of the studies, while the aortic spiral strip was used for one study.

Considering first the isolated perfused mesenteric bed: adherence to a rigid time schedule was essential for studies using

this preparation because it tended to become oedematous after several hours of perfusion with Kreb's solution and also because the sensitivity to many drugs appeared to alter after the preparation had been set up for approximately three hours.

The maximal responses elicited by the two vasoconstrictor agents noradrenaline and 5-HT were significantly greater for preparations from dietary obese rats than for those from lean controls. This was true for responses to 5-HT after 4 and 15 weeks and for responses to noradrenaline after 8, 10 and 20 weeks. The sensitivity of the mesenteric bed to 5-HT from obese rats at 4 and 15 weeks was greater than that of tissues from lean age-matched controls (as reflected in a lower ED_{50} for tissues from obese rats). In hypertension the responsiveness of the blood vessel wall to many vasoconstrictors is increased (Webb, 1984). One factor which may contribute to this enhanced reactivity is the structural change(s), resulting in an increased wall:lumen ratio of the vessel (Vanhoutte, 1986). In spontaneously hypertensive rats an increased quantity of smooth muscle has been shown to occur in various vascular structures, including the mesenteric bed (Mulvany et al, 1978). Further work has suggested that the increase in smooth muscle develops later in life, possibly as a result of the elevated blood pressure (Mulvany, 1984). However, other workers using spontaneously hypertensive rats have shown increases in smooth muscle in the mesenteric vascular bed even at the prehypertensive phase (Lee, 1985). As the dietary obese rats in the studies described here exhibited an elevation in blood pressure, it is possible that the increase in maximum responses seen to both noradrenaline and 5-HT in the mesenteric beds from these rats was due, at least in part, to a greater quantity of vascular smooth

muscle. It has been observed that a high potassium intake markedly reduced mesenteric arteriolar hypertrophy in stroke-prone spontaneously hypertensive rats, without a significant reduction in blood pressure (Tobian et al, 1986). Therefore, dietary factors may have been involved in any structural changes which occurred in the blood vessel walls of the dietary obese rats (their diet being relatively low in potassium). In future studies, histological investigations of the blood vessels from the lean and dietary obese rats could yield useful information about any possible structural differences.

McGregor and Smirk (1970) studied the mesenteric vasculature from rats with various forms of hypertension. They found that the threshold for the direct vasoconstrictor effect of 5-HT was lower, the dose-response curve steeper and the maximal response greater than the values obtained for tissues from normotensive rats. Vanhoutte (1986) suggested that the cellular mechanisms underlying the increased sensitivity to 5-HT probably do not involve an increased affinity of serotonergic receptors for 5-HT, since the inhibitory potencies of serotonergic antagonists, such as ketanserin and methysergide were comparable in blood vessels from normotensive and hypertensive rats. A possible explanation for the increased response to 5-HT is a greater mobilization of calcium from intracellular stores (Webb & Vanhoutte, 1985). This possibility is of interest when considering the mesenteric vasculature from the dietary obese rats used in these studies, as these preparations also show an increased response to raised calcium concentrations in the perfusate. The sensitivity of the mesenteric vasculature from dietary obese rats to increased concentrations of calcium and potassium in the Kreb's

solution (as reflected in higher peak plateau pressures with both '65mM potassium Kreb's' and '65mM potassium plus 3mM calcium Kreb's' and a more rapid attainment of maximum pressure plateau with the '65mM potassium plus 3mM calcium Kreb's') was greater than for tissues from age-matched lean controls after 8 weeks. No significant differences were seen after 4 weeks, therefore it appears that this increased sensitivity seen in the tissues from dietary obese rats may take time to develop. Increased calcium sensitivity of mesenteric vessels from spontaneously hypertensive rats, compared with those from Wistar- Kyoto rats (normotensive) has been shown by Mulvany (1984). The reason for this difference is unknown at present.

There were no significant differences between the responses of the isolated mesenteric vasculature taken from lean and dietary obese rats to perfusion with phenylephrine or to the subsequent doses of acetylcholine. However, a time-related increase in sensitivity to the contractile effect of phenylephrine was observed in tissues from both lean and dietary obese rats.

Secondly, considering the responses of isolated perfused hearts from lean and dietary obese rats: there were no significant differences between the mean baseline beating rates of hearts from lean and dietary obese rats after 4 or 8 weeks. This was in contrast to the faster 'in vivo' heart rates seen in obese rats compared with lean. It appears that the inotropic effect of noradrenaline is enhanced in hearts from obese rats compared with those from lean (see section 3.1.5), while the chronotropic and arrhythmogenic effects of the drug are similar for hearts from both groups.

Considering the effects of acetylcholine in the isolated perfused heart: both the duration of cardiac arrest and the recovery

time increased with time to a greater extent in hearts from obese rats than in those from lean controls, the responses to acetylcholine in hearts from the latter group showing little change with time. Thus, while the recovery time for the hearts from obese rats was shorter than for hearts from lean controls after 4 weeks, the hearts from lean rats showed the shorter recovery time after 8 weeks.

Thirdly, the single study of the response of aortic spiral strips from lean and dietary obese rats to noradrenaline showed a relative insensitivity of tissues from the dietary obese rats. However, studies using this preparation were not continued for the reasons stated earlier (see section 1.7.1c)).

In future studies, analysis of plasma levels of the catecholamines noradrenaline and adrenaline would be carried out, as the results might be of help in the interpretation of the data obtained from the 'in vitro' preparations.

4.5 THE EFFECTS OF TREATMENT WITH SELECTED ANORECTIC DRUGS OR WITHDRAWAL OF THE PALATABLE DIET IN DIETARY OBESE RATS TOGETHER WITH CONSIDERATIONS NEEDED WHEN CHOOSING DRUG TREATMENT FOR OBESITY IN MAN.

The drugs chosen were amphetamine, fenfluramine and mazindol. The reasons for choosing these particular drugs and their pharmacological actions have been discussed earlier (see section 1.4). The aims of this part of the work were : firstly, to monitor any changes in weight, blood pressure and heart rate occurring in previously obese rats following withdrawal of the palatable diet. The isolated perfused heart and mesenteric vasculature were studied in order to identify specific areas of the cardiovascular system where

changes might have occurred. The 'in vivo' and 'in vitro' values were compared with those of age-matched lean (chow-fed for whole study) and dietary obese (chow plus palatable diet for whole study) rats. Secondly, the effects of the three anorectic drugs were studied in obese rats still receiving the palatable diet and the values compared with those of the three groups mentioned above (lean, obese and diet withdrawn). The anorectic potency of the drugs has been well established (see section 1.4) and Table 3.2(iii) shows the efficacy with which they reduced food intake in this study.

Body weight gain during the first 4 weeks of the study, when all groups (except lean controls) received the palatable diet, was extremely variable (see Table 3.2(v)). A possible mechanism for this could be a seasonal effect(s), which was discussed earlier (see section 4.1). Mean weight loss during the second 4 weeks of the study was similar for the amphetamine-treated group (AMPH) and the 'diet withdrawn' group (WD). The mean weight loss for both the fenfluramine-treated group (FEN) and mazindol-treated (MAZ) group were smaller (see Table 3.2(v)). There was a wide variation in the weight lost with mazindol and amphetamine. In contrast, weight loss with fenfluramine treatment showed much less variation.

During the first 4 weeks of the study, the rise in systolic blood pressure was greater in all the groups receiving the palatable diet than in chow-fed controls (see Table 3.2(vii)). Several possible mechanisms for this greater increase in blood pressure occurring in the dietary obese rats have been discussed in section 4.2. The changes in blood pressure during the second 4 weeks of the study, expressed as 'mmHg per gram of weight lost' were as follows (mean values for each group): for WD, -0.76; for AMPH, -0.17; for FEN,

-2.34; for MAZ, +0.39. Thus, weight loss by withdrawal of the palatable diet was associated with an appreciable fall in blood pressure (see Table 3.2(vii)). However, similar weight loss achieved without withdrawal of the palatable diet, but with a daily dose of 5mg/kg amphetamine resulted in a much smaller reduction in blood pressure. This suggests that either amphetamine exerted an intrinsic hypertensive effect, which partially negated the hypotensive effect of weight loss and/or that it was the withdrawal of the palatable diet which was the most important factor for the reduction of blood pressure rather than the actual weight loss achieved. The presence of a significant negative correlation between weight change and blood pressure change (see Table 3.2(viii)) favours the suggestion that amphetamine may be exerting an intrinsic hypertensive effect and that the sensitivity to this effect and to the anorectic effect of amphetamine may be correlated (the rats which lost the greatest amounts of weight exhibited smallest reductions in blood pressure and even, in some cases, increases in blood pressure). Considering the nutrient intake (expressed as % of the intake of age-matched, untreated dietary obese rats) of AMPH and WD (see Table 4.5(1)): whilst energy and sodium intakes were similar for the two groups, the fat intake of AMPH was approximately 400% that of WD. Calcium intake of AMPH was approximately 25% that of WD and potassium intake was approximately 30% that of WD. Thus, in view of the protective or hypotensive effects proposed for a high potassium intake; the possible hypertensive effects of a high fat intake and the possible contribution of a calcium deficiency to blood pressure elevation, it appears that the nutrient balance seen after the withdrawal of the palatable diet may, in itself, contribute to the hypotensive effect

Table 4.5(1)

Nutrient intake of rats during administration of fenfluramine , mazindol , or amphetamine compared with that after withdrawal of the palatable diet. All values were calculated from single 24-hour measurements of food intake and are expressed as % of intake of age-matched dietary obese rats which had received the palatable diet for 7 weeks (see Table 3.1(1)).

	<u>WD</u>	<u>AMPH</u>	<u>FEN</u>	<u>MAZ</u>
Energy	43.93	43.21	75.13	45.30
Protein	116.58	48.19	76.68	47.15
Fat	19.11	96.18	124.84	82.80
Carbohydrate	41.88	28.61	59.48	34.06
Fibre	46.39	26.80	64.95	35.05
Sodium	19.92	24.44	65.25	40.94
Potassium	165.92	55.92	74.04	50.22
Calcium	300.64	72.88	79.42	55.02

Key

WD = palatable diet withdrawn

AMPH = amphetamine 5mg/kg daily

FEN = fenfluramine 10mg/kg daily

MAZ = mazindol 4mg/kg daily

seen. Whereas the nutrient balance resulting from amphetamine administration, with the continued availability of the palatable diet (albeit with a reduced consumption of most items), would appear to be less conducive to blood pressure reduction (see section 1.5 for discussion of the effects of various dietary factors on blood pressure).

The reduction in blood pressure achieved with fenfluramine was considerably greater than expected from the modest weight loss. It, therefore, appears that fenfluramine may have exerted an intrinsic hypotensive effect, in addition to its anorectic action. The dietary intake of fat, sodium and carbohydrate was greater, and the intake of calcium and potassium lower for FEN than for WD (see Table 4.5(1)) and therefore it was unlikely that the nutrient balance played a significant part in the greater hypotensive effect seen with fenfluramine.

The final blood pressure measurements (after 4 weeks of drug administration) were made for amphetamine-treated and mazindol-treated rats on the last day of drug administration. However, in order to obtain values for the blood pressure changes occurring after 4 weeks of fenfluramine administration, it was necessary to take the blood pressure readings 18 hours after the cessation of fenfluramine administration. Attempts to obtain tail cuff blood pressure readings during the period of fenfluramine administration yielded only a few faint, unclear readings from some rats in the group and no measurable traces from some others. It was only possible to obtain clear readings from two or three rats out of the twelve in the group. However, the readings obtained from these rats were low and similar to those obtained for the group as a whole after fenfluramine

withdrawal. In order to obtain a clear blood pressure reading using the Narco programmable electrospychomanometer, a good flow of blood through the tail, resulting in a strong pulse in the caudal arteries is essential. This is because the systolic blood pressure value is taken as the mean of the 'cut-off' pressure at which the pulse ceases and the 're-start' pressure at which the pulse restarts (see Fig.9 for specimen blood pressure trace). During administration of fenfluramine, only a very faint pulse was discernible in the traces obtained from most of the rats. One possible explanation for this which was considered was that fenfluramine might have been exerting a hypothermic effect, resulting in constriction of the caudal blood vessels as an attempt to reduce heat loss. A significant reduction in body temperature in rats was reported after administration of fenfluramine in a study by Jonsson and Gunne (1972). However, rectal temperatures of the fenfluramine-treated rats in the studies described here (during the period of administration of fenfluramine) were recorded both before and after the 10 minute pre-warming period (the warming box was maintained at $36 \pm 1^{\circ}\text{C}$) and were found to be within the range $36.8-39.0^{\circ}\text{C}$. A standard value for the body temperature of the rat is given by Waynforth (1980) as 38.1°C , therefore the values obtained for the fenfluramine-treated rats were not abnormally low. Thus, it does not appear that fenfluramine exerted a significant effect on the body temperature of the rats in this study.

Another possible explanation for the faint pulse in the tail of the fenfluramine-treated rats, which was considered, was that fenfluramine might have been exerting a vasoconstrictor effect on the caudal blood vessels. However, a study by Jespersen and Bonaccorsi

(1969) showed no vasoconstrictor effects of fenfluramine in the isolated tail artery of the rat. On the contrary, they found that perfusion with fenfluramine abolished the constrictor effect of tetrabenazine and to a smaller extent that of noradrenaline, in this preparation. It is therefore apparent that further work is required to investigate the effects of an anorectic dose of fenfluramine (10mg/kg daily) on the blood flow in the tail of the rat.

The effects of mazindol upon both weight change and blood pressure change were extremely variable. However, it was noted that a significant negative correlation existed between changes in weight and changes in blood pressure, similar to that seen in the amphetamine-treated group. Therefore, it is possible that the sensitivity of the rats to the anorectic action of mazindol correlated with some hypertensive effect. The dietary intake of the mazindol-treated (MAZ) rats was similar to that of the amphetamine-treated (AMPH) rats, though with a slightly higher sodium intake (see Table 4.5(1)). Therefore, the slight increase in blood pressure seen in MAZ, despite a mean reduction in weight for the group, may have been due in part to dietary factors, but was probably also due to intrinsic hypertensive effects of mazindol. The simultaneous increase in both blood pressure and heart rate (see below) seen with mazindol suggests that it may be having a sympathomimetic effect.

The heart rate of both lean and dietary obese rats increased slightly during the second 4 weeks of the study (see Table 3.2(x)). Withdrawal of the palatable diet resulted in a decrease in heart rate. Administration of amphetamine and fenfluramine both brought about significantly greater reductions in heart rate than that

occurring with dietary restriction. Mazindol, however, produced a large increase in heart rate.

It is appropriate to consider next the effects of the different treatments on the responses of the isolated perfused heart preparation. Neither the baseline beating rate, nor the response of the beating rate to doses of noradrenaline, showed any consistent significant differences between lean, obese and the various 'weight reduced' groups. However, the contractility response to doses of noradrenaline (expressed as % baseline contractility) showed a greater sensitivity in hearts from both fenfluramine-treated (FEN) and mazindol-treated (MAZ) rats. The arrhythmogenic effect of noradrenaline showed no significant difference between groups. Neither were there any consistent trends in the responses of the isolated hearts to acetylcholine for any of the groups.

The maximal response of the isolated perfused mesenteric vasculature to noradrenaline was significantly smaller for tissues from all 'weight reduced' animals than for those from the age-matched dietary obese group. The greatest depression in maximal response was seen in the mesenteric vascular beds from MAZ and WD.

When considering the overall cardiovascular function of the different groups of 'weight reduced' rats, it is important to consider the following facts: systemic arterial blood pressure is dependent upon cardiac output and peripheral resistance ; and cardiac output is proportional to heart rate and contractility. The reduction in blood pressure seen in group WD may have been due partly to a reduced cardiac output, as a result of a reduced heart rate (seen 'in vivo' and unchanged contractility response (as determined 'in vitro')); and also partly to a decrease in peripheral resistance

which may have resulted from the decrease in vascular responses to noradrenaline (compared to obese rats), as seen 'in vitro' in the isolated perfused mesenteric bed. This decreased maximal response to noradrenaline in the mesenteric bed may have been partially due to a reduction of any smooth muscle increase which might have developed as a result of the increased blood pressure seen in the dietary obese rats (see section 4.4). The reduction in both heart rate and blood pressure may have been partly due to the reduction in sympathetic nervous system activity, which has been reported to occur as a result of a reduction in food (especially carbohydrate) intake. Measurements of plasma catecholamine levels in future studies would be of help in the interpretation of this data and also data from the other groups.

The smaller mean reduction of blood pressure seen in AMPH compared with WD may have been the result of antagonism between the sympathomimetic effects of amphetamine and the reduction in sympathetic stimulation occurring as a result of decreased food intake. The response of the isolated mesenteric vasculature from AMPH was greater than for tissues from WD. Thus, it appears that if the reduction in the maximal response seen in mesenteric vascular tissues from WD was the result of vascular smooth muscle reduction, there may have been less reduction of the smooth muscle in tissues from AMPH. The report by Tobian et al (1980) that a high potassium diet is beneficial in the reduction of vascular smooth muscle hypertrophy in hypertension is of interest, considering that the potassium intake of group WD was approximately three times that of AMPH.

The marked hypotensive and negative chronotropic effects observed after treatment with fenfluramine suggest that the drug may have had a direct sympatholytic action. Similar effects have been

observed by Chernow et al (1983) in moderately obese, normotensive men. They found that plasma noradrenaline levels had declined after one week of fenfluramine treatment and remained depressed throughout the rest of the treatment period (4 weeks in total). However, they found no significant correlation between the changes in supine plasma noradrenaline concentrations and changes in mean arterial pressure from baseline values to those at either one or four weeks. There is evidence that fenfluramine may exert its sympatholytic effect by central presynaptic α_2 -adrenoceptor activation (Chernow et al, 1973). The increased sensitivity of the isolated heart to the contractility effect of noradrenaline may reflect 'up regulation' of beta-adrenergic receptor density in the heart. Similarly, the marked tachycardia and slight increase in blood pressure associated with mazindol in the dietary obese rats may be the result of sympathomimetic activity and the decreased maximal response to noradrenaline in the mesenteric bed may reflect a 'down regulation' of noradrenergic receptor density in this tissue. Alternatively, it is possible that the increase in heart rate and small rise in blood pressure seen with mazindol reflect excessive reflex tachycardia, occurring as a result of reduced peripheral resistance (note the greatly reduced maximal response to noradrenaline in the mesenteric bed). Further studies involving the monitoring of plasma noradrenaline and adrenaline levels and also the responses of other vascular tissues 'in vitro' would be necessary to try to clarify this point. It is of interest to note that the isolated hearts from the mazindol-treated group exhibited greatly enhanced sensitivity to the effects of noradrenaline on contractility. If mazindol had produced increased sympathetic stimulation 'in vivo', then this increase in

sensitivity to noradrenaline seen 'in vitro' would not have occurred by the same mechanism as that proposed for the fenfluramine group (that is, 'up regulation' of receptor density). Cardiovascular side-effects of mazindol, including atrial fibrillation, have been reported in man by Bradley et al (1974), suggesting that it should probably be avoided in obese patients with overt cardiac disease.

When selecting the most appropriate treatment strategy for obesity, it is important to consider why treatment is necessary. This may be for purely aesthetic or social reasons, with no underlying health problems; or it may be as 'preventative medicine' to avoid the possible health complications associated with obesity; thirdly, it may be as a method of treatment for an overt health problem such as hypertension or Type II diabetes mellitus; or it may be for any combination of these reasons.

If drug treatment is to be used as an adjunct to dietary restriction, the choice of drug is important. The drug should not have any intrinsic actions which might negate possible benefits of weight loss. As stated earlier, mazindol is not considered appropriate for the treatment of patients with any cardiac dysfunction. Chronic mazindol treatment has also been shown to have a hyperinsulinaemic effect (Sirtori et al, 1971) and should therefore be used with caution in any patient who already exhibits any degree of hyperinsulinaemia or carbohydrate intolerance. The effects of mazindol on blood pressure of rats, in the studies described here, also suggest that the drug should be used only with careful monitoring in any obese patient with hypertension. Mazindol has been shown to raise metabolic rate in rats (Wyllie et al, 1984) and to

prevent the fall in energy expenditure normally associated with a reduction in food intake. This makes it a useful drug for use in patients who lose little weight even when adhering to a low calorie diet. The hypotensive and hypoglycaemic effects of fenfluramine (see section 1.4.3) make it particularly suitable for the treatment of obese patients with mild hypertension and/or hyperglycaemia. Any regular treatment normally given for these conditions may have to be re-evaluated during treatment with fenfluramine. However, the adverse effects of fenfluramine, which may include sedation and depression (Craddock, 1978), make it unsuitable for some patients.

4.6 THE USE OF A DIETARY SURVEY TO STUDY CAFFEINE INTAKE IN DIETERS AND NON-DIETERS.

As stated in section 2.5, the main aim of the survey was to determine whether or not dieters tend to increase their consumption of coffee (and hence their intake of caffeine) when dieting. The results of the survey showed that a proportion of dieters (25%) did, indeed, increase their intake of coffee when dieting. Whilst dieting, dieters consumed significantly more coffee (expressed as cups per day) than non-dieters (4.48 ± 0.73 and 3.21 ± 0.44 respectively), $p < 0.01$. This was mainly due to the greater coffee consumption, whilst dieting, of the male dieters (5.83 ± 1.19). The male dieters were the group with the greatest body mass index (BMI) (25.82 ± 1.17), which fell into Grade I category obesity (Garrow, 1988). Therefore, the known cardiovascular risks associated with excess body weight might compound any possible risks from the greater caffeine intake of this group. As men are known to have a generally greater risk of cardiovascular disease than women, it appears that this group may be

the most at risk for cardiovascular disorders.

The BMI of the dieters (male plus female) was greater than that of the non-dieters (23.30 ± 0.62 and 21.51 ± 0.36), both values falling within the Grade 0 category of obesity (Garrow, 1988). However, when the dieters were subdivided into male and female, it was seen that while the BMI of female dieters was well within the Grade 0 category (22.47 ± 0.63), that of the male dieters fell within the Grade 1 category. This observation tends to imply either that female dieters were more successful in their attempts to lose weight than male dieters and so reached and maintained a normal body weight, whilst the male dieters remained overweight; or alternatively that female dieters dieted when they were not actually overweight.

It was found that smokers drank significantly more cups of coffee per day than non-smokers (4.64 ± 0.87 and 3.23 ± 0.41), this difference being mainly due to the greater amount of coffee consumed by the male smokers (5.71 ± 1.08) compared with male non-smokers (2.96 ± 0.43). Smoking is a proven cardiovascular risk factor (United States Public Health Service, 1964). As mentioned earlier, men tend to be at greater risk from cardiovascular disease than women, therefore male smokers with a greater coffee intake may be particularly at risk. A significant correlation was found between the number of cigarettes smoked daily and the number of cups of coffee consumed daily, even though the group of subjects was small ($n=10$) (see Fig.22). The correlation became stronger when the total number of cups of tea plus coffee were used, rather than coffee alone (see Fig.23). Coffee drinking has been found to be strongly associated with current smoking habits in other studies also (La Croix et al, 1986).

Overall, women consumed more cups of tea per day than men (2.40 ± 0.39 and 1.28 ± 0.31), though the mean intake of tea for all subjects was only approximately half that of coffee (1.82 ± 0.26 and 3.55 ± 0.38). This is a surprising result for a survey of a group of U.K. subjects, because tea consumption (ounces per week) for the population of the U.K. as a whole is reported as more than twice that of coffee (1.74 and 0.72) (Ministry of Agriculture, Fisheries and Food, 1986). Even after allowing for the different methods of preparation this still shows a larger intake of tea than coffee overall.

There was a weak but significant correlation between BMI and alcohol intake (number of 'standard drinks' per day), with a correlation coefficient (r) of 0.2788, ($p < 0.05$). However, when dieters (not dieting) were considered separately, this correlation became much stronger, $r = 0.6773$ (see Fig.24). However, this may not mean that the higher alcohol intake was a contributory factor in the greater value of BMI, as many other factors must be considered (such as total energy intake and energy expenditure).

It was of interest to note that subjects who consumed 1 or 2 cups of coffee daily had a significantly lower mean BMI than either non-coffee drinkers or subjects who consumed 3 or more cups of coffee daily. Caffeine (25mg/kg) at a dose equivalent to approximately 4 cups of coffee per day in man, resulted in a reduced rate of weight gain in lean Wistar rats compared to controls who did not receive caffeine, but a higher dose of 50mg/kg resulted in an increased rate of weight gain in both lean and dietary obese rats (see section 3.6). The possible mechanisms for these effects are discussed below (see section 4.7).

4.7 THE EFFECTS OF CAFFEINE (25MG/KG AND 50MG/KG), GIVEN WITH OR WITHOUT A PALATABLE DIET, IN FEMALE WISTAR RATS.

The doses of 25mg/kg and 50mg/kg were selected as equivalent to moderate and high caffeine intake in man (see section 4.6). Yeh et al (1986) used doses of 25mg/kg and 100mg/kg (by subcutaneous injection) which they stated were equivalent to the consumption of 4 and 16 cups of coffee by a 70kg human subject (assuming that the caffeine content of a cup of coffee is approximately 85mg). The conversion of doses from rats to humans was based upon metabolic body weight ($\text{kg}^{0.75}$), (Kleiber, 1961). 100mg/kg was felt to be an excessive dose to represent even a high 'normal' coffee intake in man, thus 25mg/kg and 50mg/kg were selected for the experiments described here. These doses have been shown to produce neuroendocrine effects in rats (Spindel et al, 1980 and 1983).

Firstly, considering the effects measured 'in vivo' on weight gain and food intake. The lower dose reduced the weight gain of the lean rats during the study but increased that of the dietary obese rats, whereas the higher dose increased the weight gain of both lean and dietary obese rats. Results of analysis of food intake in the lean chow-fed rats showed that changes in food consumption may have contributed to the effects on weight gain in these animals. Chow intake was almost unchanged (slightly reduced) with 25mg/kg caffeine but increased with 50mg/kg caffeine ($p < 0.001$). Caffeine has been shown to cause the release of immunoreactive beta-endorphin and to increase serum beta-endorphin-like immunoreactivity in both rats (Arnold et al, 1982) and man (Spindel et al, 1984). Beta-endorphin-like immunoreactivity in cerebrospinal fluid was not increased with

caffeine (Arnold et al, 1982). Activation of the beta-endorphin-epsilon opioid receptor in the rat has been shown to enhance feeding (Morley & Levine, 1982a). It is therefore possible that the effect of caffeine on this endogenous opioid peptide may alter food intake in the rat. The apparent lack of effect upon food intake in the dietary obese rats (see section 3.6.4) may be due to the fact that these single measurements were carried out during the fourth week of the study, by which time tolerance to the effects of caffeine may have developed. Tolerance to many of the effects of caffeine has been shown to occur: in the cardiovascular system within 1 to 4 days in man (Robertson et al, 1981); and in rats to the elevation of serum corticosterone levels within 6 days (Spindel et al, 1983) and partial tolerance to the depression of serum thyrotropin (TSH) levels within 13 days (Spindel et al, 1983). It is therefore reasonable to suppose that some degree of tolerance would occur to the effects of caffeine upon serum beta-endorphin-like immunoreactivity and food intake. However, the monitoring of food intake of the chow-fed rats was carried out during the first 10 days of the study, when tolerance was less likely to have developed. Comprehensive monitoring of food intake of both lean and dietary obese rats during treatment with 25mg/kg and 50mg/kg caffeine would be required to clarify this point. However, assuming that the greater weight gain seen in the dietary obese rats, with both doses of caffeine, is due, at least in part, to an increased food intake during the early part of the experiment, the following theory could account for the different effects of the two doses of caffeine on weight gain in lean and obese rats. Activation of the beta-endorphin-epsilon opioid receptor caused by caffeine would tend

to enhance feeding. The dietary obese rats already have a greater motivation to eat than the chow-fed rats (hedonic influence) and may respond to lower doses of caffeine, and therefore might increase their food intake when given both 25mg/kg and 50mg/kg caffeine. The lean rats appear to have less motivation to eat and therefore might require higher doses of caffeine to induce enhancement of food intake. Hence the lack of response to the 25mg/kg dose.

The effects of high doses of caffeine in the rat (50mg/kg being equivalent to approximately 8 cups of coffee) appear to differ from those in man, as a daily dose of 500-600mg caffeine (approximately 6 cups of coffee) may produce a condition known as 'caffeinism', one of the symptoms of which is loss of appetite (Abbott, 1986).

The slight reduction in weight gain seen in the lean rats receiving 25mg/kg caffeine might be attributed, partly, to the small, though non-significant decrease in food intake seen and partly due to the stimulation of metabolic rate which has been shown to occur with caffeine treatment. A study by Jung et al (1981) showed that 4mg/kg caffeine, given orally or intravenously (equivalent to approximately 3 cups of coffee in a 70kg subject) to lean and obese subjects caused a rapid increase in metabolic rate, which was sustained over the two hour test period. All the subjects were habitual coffee/tea drinkers, with a normal daily intake of approximately 300mg caffeine. Another study by Acheson et al (1980) also showed an increase in metabolic rate with doses of 4mg/kg and 8mg/kg caffeine in both lean and obese subjects. However, the normal daily caffeine intake (if any) of these subjects was not stated. Any rise in metabolic rate occurring in the other three caffeine-treated groups of rats (LC50, DC25, DC50) may have been more than compensated for, in terms of energy balance, by

increases in food intake.

Secondly, considering the effects upon 'in vivo' heart rate and blood pressure together with the 'in vitro' cardiovascular tissue preparations. The time-related increases in blood pressure were greater in all the dietary obese groups (both with and without caffeine) than in the corresponding lean groups. The lower dose of caffeine did not significantly effect the time-related increase, however, the 50mg/kg dose reduced the increase in both lean and obese rats. This result is in agreement with the clinical study by Periti et al (1987), which showed a 'dose'-dependent reduction in both systolic and diastolic blood pressure per cup of coffee per day. The 25mg/kg dose reduced the time-related increase in heart rate which was seen in both lean and dietary obese rats, though this was only significant for the lean animals. The 50mg/kg dose reduced the time-related increase in heart rate seen in the obese but not in the lean rats. Thus, the effects of caffeine on heart rate and blood pressure appear to reflect a relative insensitivity to caffeine in the obese rats. The reduction in the time-related rise in blood pressure was only seen at the higher dose in the obese rats, though a small (non-significant) reduction was seen in the lean with the lower dose. A large reduction in the time-related increase in heart rate was seen in the lean rats with the lower dose, whereas a significant reduction was only seen with the higher dose in the obese rats. However, in both lean and obese rats caffeine tended to cause a reduction of the time-related increases seen in both blood pressure and heart rate.

One possible mechanism by which caffeine could induce this effect is by a reduction of serum thyrotropin (TSH) levels (Spindel

et al, 1980 and 1983). This could, in turn, result in a reduction in serum triiodothyronine (T_3) and thus a reduction in sensitivity to pressor mechanisms and result in a fall in blood pressure (T_3 , in the rat, has been shown to increase beta-adrenergic receptor numbers, which would increase sensitivity to pressor mechanisms (Williams et al, 1977)). A 50mg/kg challenge dose of caffeine was found to cause a reduction in serum TSH by approximately 60%. This inhibition of TSH by caffeine does not appear to be by adenosine receptor blockade, as adenosine agonists (adenosine and N^6 -2-(L-phenylisopropyl)-adenosine) did not affect serum TSH. In earlier studies by Spindel et al (1980) caffeine was shown to reduce serum TSH in a dose-dependent manner, the reduction persisting for 1 to 6 hours after the injection of caffeine. The reduction in serum TSH was followed by a reduction in serum T_3 and T_4 4 hours after caffeine administration. Daily administration of 50mg/kg caffeine (by intraperitoneal injection) for 6 to 13 days produced a time-related tolerance to the effects of a further challenge dose of 50mg/kg caffeine, although TSH values were still reduced by approximately 30% by the challenge dose even after 13 days chronic caffeine administration (Spindel, 1983). Serum caffeine levels of 15-18 μ g/ml (equivalent to 0.08-0.09 mM) , which could be achieved by a single dose of caffeine of approximately 20mg/kg, were found to be the lowest at which a depression in TSH levels were seen. Serum levels of 15 μ g/ml or higher can be obtained in man after 2 to 3 cups of coffee (or at least 250mg caffeine). However, serum caffeine levels were not determined in the studies reported here. In further work this should be monitored. It seems unlikely that such levels would be obtained with the lower dose of caffeine, as the total daily dose was only 25mg/kg and the rats were

unlikely to consume almost all of their daily fluid intake at one time. However, it is possible that the required levels of caffeine might have been achieved with the high dose. Low doses of caffeine may stimulate the thyroid slightly, as hormonal stimulation of the thyroid is mediated, in part, by c-AMP (cyclic adenosinemonophosphate) (Gilman & Rall, 1966), but this effect would be masked by the inhibition of TSH occurring with higher doses. Monitoring of serum T_3 and TSH levels would be important in further studies.

Caffeine might also have caused changes in cardiovascular function by its effects upon calcium mobilization within the cardiac myocytes. Caffeine (1-20mM) has been shown to produce a negative inotropic effect in cultured chick embryo ventricular cells by decreasing the sarcoplasmic reticulum (SR) calcium (Ca^{2+}) uptake and/or an increase in SR Ca^{2+} release that eventually depleted the SR of Ca^{2+} (Rasmussen et al, 1987). Thus, a reduction in the contractility of the heart 'in vivo' might be partly responsible for the reduction in the increase of systolic blood pressure observed in these studies, by a reduction of cardiac output, provided the effect on calcium mobilization could be achieved with lower concentrations of caffeine than those used by Rasmussen et al (1987), as the maximum plasma concentration of caffeine likely to have been achieved in these rat studies would have been approximately 0.1mM.

Another mechanism by which caffeine might have its cardiovascular effects is by a direct blockade of the adenosine receptor. Adenyl compounds may cause dilation of blood vessels but can also cause constriction, as is seen in the renal artery of the rat (Hrdina et al, 1967 and 1970). Adenosine, in an increasing dose of 0.04 to 0.16mg/kg/minute, has been shown to induce a rise in

systolic blood pressure of 17mmHg and in heart rate of 33 beats per minute, and also a small fall in diastolic blood pressure of 4mmHg in healthy, normotensive male volunteers (Smits et al, 1987). A positive inotropic effect is sometimes seen after the initial negative inotropic response of the heart to adenyly compounds (Gillespie, 1934). Interaction of caffeine with endogenous adenosine may therefore be responsible for the reduction in the time-related increases in heart rate and blood pressure observed in the studies described here (by inhibiting any adenosine-induced vasoconstriction, and increases in systolic blood pressure and heart rate).

The isolated tissue preparations (heart and mesenteric bed) were studied to try to identify specific areas of the cardiovascular system which caffeine might be affecting. The isolated perfused heart was the obvious choice of 'in vitro' preparation for studying cardiac changes and the mesenteric vasculature was chosen partly because it had already been used in studies of obesity and weight reduction (see sections 3.1.7 and 3.2.6) and partly because long-term administration (up to 117 weeks) of caffeine (0.102% in the diet) has been shown to produce periarteritis nodosa-like lesions in the mesenteric vessels of the rat (Johansson, 1981). This condition was sometimes so severe that it caused death of the animal, due to massive intra-abdominal haemorrhage as a result of rupturing of the damaged vessels.

The responses of the isolated perfused heart to three doses of noradrenaline, in terms of percentage of baseline contractility, were greater for hearts from obese (untreated) than lean (untreated) rats (see Fig.29). Chronic administration of both 25mg/kg and 50mg/kg caffeine reduced the responses of isolated hearts from both lean and obese rats in a dose dependent manner, such that the responses of

hearts from obese caffeine-treated rats (both doses) were lower than for hearts from untreated obese rats and similar to those of hearts from lean rats (see Fig.29).

The effects of the same three doses of noradrenaline on the beating rate of the isolated perfused hearts were dose-dependent for all groups and showed little difference between the groups, although the responses of the hearts from the lean rats, which had received 25mg/kg caffeine, tended to be smaller than those of hearts from all the other groups. This is of interest as this dose of caffeine produced a large reduction in 'in vitro' heart rate in the lean rats.

Although caffeine has been shown to promote arrhythmogenesis in humans (Dohmeyer et al, 1983), there was no significant difference in the occurrence of arrhythmias after doses of noradrenaline in isolated hearts from lean or obese rats at either dose level of caffeine.

After 4 weeks of study (chow with or without the palatable diet) there was little difference between the maximal response of the isolated perfused mesenteric vasculature from lean and obese rats (untreated). (The greater maximal response of the mesenteric vasculature from dietary obese rats was not seen until 8 weeks, see Fig.12). However, both doses of caffeine reduced the maximal responses to noradrenaline of the mesenteric vasculature from both lean and obese rats, though dietary obesity attenuated the reduction seen to some extent (see Fig.30).

Thus, it appears that, in general, caffeine treatment tended to reduce the cardiovascular effects of dietary obesity, those effects being increased time-related rise in both heart rate and systolic blood pressure. It is therefore tempting to speculate that caffeine

may affect one or more of the parameters associated with the cardiovascular changes occurring in dietary obesity. Increased carbohydrate intake and hyperglycaemia have been proposed as possible contributory causes of the apparent enhancement of sympathetic nervous system activity seen in dietary obesity (see section 4.2). Three lines of evidence suggest that caffeine might have effects on plasma glucose levels. Firstly, high affinity A_2 -adenosine receptors (as defined by Daly, 1985), linked to adenylate cyclase in a stimulatory fashion, have been identified in rat liver plasma membranes (Londos et al, 1980). Secondly, glucose production is stimulated by adenosine in the perfused rat liver (Buxton et al, 1986) and by adenosine analogues in isolated rat hepatocytes (Hoffer & Lowenstein, 1986); and thirdly a negative correlation between plasma glucose concentration and the caffeine content of the diet has been observed in rats (Wurzner et al, 1977) and in some studies caffeine improves glucose tolerance in man (Feinberg et al, 1968). Further studies involving regular monitoring of carbohydrate intake, plasma glucose concentration and plasma caffeine concentration would be required to investigate the effects of caffeine on the handling of carbohydrate in this dietary obese rat model.

4.8 SUMMARY OF SUGGESTIONS FOR FUTURE WORK

From the foregoing discussion, it is apparent that the measurement of various parameters is required to clarify a number of the findings. Measurements of plasma noradrenaline and adrenaline levels, as indicators of sympatho-adrenal activity, should be considered in further studies using lean, dietary obese and weight-reduced rats.

The use of a "low salt" palatable diet, to distinguish between the effects of weight and sodium intake on blood pressure, would also be useful. The monitoring of plasma triiodothyronine (T_3) and thyrotropin (TSH) levels would be of interest in further studies of dietary obesity with associated elevation of blood pressure and also in any further studies using caffeine. In addition, measurement of plasma insulin levels would be required to further investigate the possible contribution of this hormone to the raised blood pressure associated with obesity.

The determination of blood calcium levels would also be a priority in further studies because of the low calcium content of the palatable diet compared with laboratory chow and the possible role of calcium in mechanisms of blood pressure control. Supplementation of the calcium and potassium intakes of the dietary obese rats to levels similar to those of the chow-fed controls would be considered in future studies. In addition, the effects of calcium channel blocking drugs and calcium ionophores would be studied to clarify the mechanism(s) by which calcium may affect cardiovascular function.

Various organs and tissues of the lean and dietary obese rats (such as heart, liver, kidneys and mesenteric vascular bed) would be weighed and full histological analyses carried out to assess any structural changes occurring in the dietary obese rats.

In order to further investigate the possibility that caffeine may have been acting, at least in part, via β -endorphinergic mechanisms, the use of opiate antagonists, such as naloxone, together with caffeine would be considered in future experiments.

APPENDIX 1

Nutrient content of the foods used (all values per 100g)

	<u>Luncheon</u>	<u>Rice</u>	<u>Digestive</u>	<u>Chocolate</u>	<u>Chow</u>
	<u>Meat*</u>	<u>Krispies*</u>	<u>Biscuits *</u>	<u>(milk)*</u>	<u>**</u>
Energy (kcal)	313	372	471	528	285.5
Protein (g)	12.6	5.9	9.8	5.7	18.1
Fat (g)	26.9	2.0	20.5	29.3	2.4
Carbohydrate (g)	5.5	88.1	66.0	65.0	57.0
Fibre (g)	3.5	4.5	5.5	neg.	3.6
Sodium (mg)	1050	1110	440	118	280
Potassium (mg)	140	160	160	420	750
Calcium (mg)	15	7	110	220	800

* Paul & Southgate (1978)

** CRM brand laboratory chow (pellets), values from manufacturers data sheet.

APPENDIX 2

Fatty acid content of the palatable food items (g per 100g food) *.

	<u>Luncheon</u>	<u>Rice</u>	<u>Digestive</u>	<u>Chocolate</u>
	<u>Meat</u>	<u>Krispies</u>	<u>Biscuits**</u>	<u>(milk)</u>
Total saturated	10.13	0.47	11.64	17.67
Total monounsaturated	12.61	0.50	7.98	9.70
Total polyunsaturated	2.30	0.72	2.42	1.06

* Paul, Southgate & Russel (1980)

** Value for "sweet short biscuits" used.

APPENDIX 3

The average caffeine content of popular beverages (mg per 6oz cup)

'Brewed' coffee	100-150
Instant coffee	60-80
Tea	40-100
Cola drinks	17-55
Decaffeinated coffee	3-5

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UNITS AND DEFINITIONS USED

Heart rate ('in vivo' and 'in vitro') - beats per minute

Systolic blood pressure / perfusion pressure - mmHg

Energy - 'calories' (1 'calorie' = 1kcal = approximately 4.27 KJ)

Statistical significance accepted at $p < 0.05$

n.s. = not significant at $p < 0.05$

AMP = adenosine monophosphate

cAMP = cyclic adenosine monophosphate

$\text{Na}^+ - \text{K}^+ - \text{ATPase}$ = sodium-potassium adenosine triphosphatase

5-HT = 5-hydroxytryptamine

BMI = Body Mass Index $\{W \text{ (kg)} / H^2 \text{ (m}^2)\}$

Weight: g (rats) or kg (man)

'dieter' = subject who had dieted

'non-dieter' = subject who had not dieted

tea/coffee intake - cups per day

mean values expressed as mean \pm standard error of the mean (unless stated otherwise)

UNITS AND DEFINITIONS USED continued:

'palatable diet' - daily quantities per cage of 6 rats :

80g Rice Krispies

40g digestive biscuits

40g luncheon meat

40g chocolate

- in addition to laboratory chow and water

Time periods stated (weeks) refer to the length of time from the start of the study that the rats have received laboratory chow with or without the palatable diet (see above), unless otherwise stated.

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